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OSSEOUS FINDINGS IN CHRONIC RENAL INSUFFICIENCY IN ADULTS *

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Now that the skeletal and renal changes which follow in the wake of primary, or idiopathic, parathyroid hyperfunctioning [1] are well understood, increasing attention is being paid to the fact that chronic renal insufficiency may itself be the point of departure for the development of profound pathologic changes in the parathyroid glands and skeletal system. In primary hyperparathyroidism, the parathyroid abnormality usually consists of an adenoma (limited as a rule to a single gland) but sometimes of hyperplasia involving all (theoretically four) parathyroids.[2] The parathyroid abnormality secondary to the metabolic alterations induced by chronic renal insufficiency also involves the theoretical four parathyroids.[3-6] However, the histological appearance of the hyperplastic glands in this group is different from that in primary hyperparathyroidism upon the basis of parathyroid hyperplasia.[7]

In some instances of chronic renal insufficiency the secondary parathyroid hyperplasia reaches such proportions as to leave no doubt that the total clinical picture has been complicated by the hyperfunctioning of these glands. This hyperfunction, in turn, may instigate further damage to the kidneys and may also exert its influence on the osseous tissues, as in primary hyperparathyroidism. Indeed, the skeletal changes may even come to occupy the foreground of the clinical picture. Thus, in childhood, protracted renal insufficiency plus "secondary" hyperparathyroidism

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form the basis for the clinicopathologic picture somewhat inaccurately designated as "renal rickets." [8] There have been reported also a few cases of chronic renal insufficiency in adults in which the parathyroid hyperplasia was as pronounced as that associated with "renal rickets" and in which there were also very extensive osseous changes.[9-12] Altogether, the skeletal changes occurring in these adult cases may be regarded as the adult counterpart of those occurring in "renal rickets," and, in both, a condition of secondary hyperparathyroidism (due to the secondary parathyroid hyperplasia) is evidently partially responsible for the evolution of the osseous changes.

The object of this paper, however, is to stress the fact that osseous lesions of a much milder type than those of the aforementioned group can almost regularly be found in adult cases of prolonged renal insufficiency. Furthermore, we shall show that these bone changes may be associated with only mild or moderate parathyroid hyperplasia and even, rarely, with no gross or microscopic parathyroid hyperplasia, so that it seems plausible to explain their development on another basis than secondary hyperparathyroidism. Such findings had been previously described only by Rutishauser and co-workers [13-16] who, in a series of papers published between 1935 and 1938, have called attention to the regular occurrence of osseous changes in adults with chronic renal insufficiency as well as in animals with experimentally produced renal insufficiency. They too question the rôle of the parathyroids in the genesis of these mild changes and stress the importance of the acidosis which is constantly present.

Observations

The present study is based upon the clinical and postmortem data on 12 cases of chronic renal insufficiency in adults. The ages of the subjects ranged from 39 to 76 years. The underlying renal damage consisted either of chronic glomerulonephritis, or chronic ascending pyelonephritis, pyonephrosis or hydronephrosis, often secondary to hypertrophy of the prostate. In all instances the state of renal insufficiency had evidently existed for some time. Furthermore, in all of them, chemical examination of the blood revealed nonprotein nitrogen retention, and hyperphospha-

temia, hypocalcemia, and lowered CO_2-combining power of the serum.

The skeletal alterations in these cases could be classified as slight or only moderately advanced (detectable only microscopically), and far advanced (discernible even on gross examination of the bones).

Slight or Moderately Advanced Changes

As already implied, when the changes were of these degrees (as they usually were), the bones appeared normal grossly. The milder the osseous changes, the more likely they were to be restricted to spongy trabeculae, the trabeculae of vertebral bodies showing them if they were to be found at all. When slight (Fig. 1), the changes consisted of the presence of scattered Howship's or resorption lacunae on the surfaces of the spongy trabeculae and even on the walls of their vascular canals. These lacunae were usually found filled with connective tissue cells and often also presented some multinucleated osteoclasts. Here and there, an erosion space showed some evidence of repair in the form of osteoblasts lining its surface, but there was no significant evidence of bone formation.

When the changes were definitely more advanced (Fig. 2), the manifestations included not only the formation of Howship's lacunae here and there on the trabeculae but also the encirclement and extensive perforation of the trabeculae by tracts of connective tissue. In addition, the cortical bone showed changes, in the form of enlargement and erosion of haversian canals. Side by side with these resorptive changes there could already be noted, in these cases, contrasting changes of a reparative nature, in the form of new-bone apposition upon the spongy trabeculae. However, in these moderately advanced cases, the reparative process was as yet slight, so that the bones as a whole still tended toward porosis.

Far Advanced Changes

Occasionally, and specifically when the renal insufficiency had been very protracted and had fluctuated, the architecture of the spongy bone was found altered, even grossly. In these cases, the

spongiosa was close-meshed and the trabeculae were thickened and distorted, and altogether the condition amounted to an osteosclerosis (Figs. 3 and 4). Microscopically, it could be observed that this osteosclerosis had developed through gradual accretion of new bone, reparative processes evidently having predominated over resorptive processes in the alternation between the two, which must have been going on for a long time. Indeed, what amounted to a "renal" osteosclerosis was present in 3 of our cases and was very pronounced in 1 of them, in which the renal disease was of 12 years' standing.

Parathyroids

In all but 1 case there was either moderate gross hypertrophy of the parathyroid glands with microscopic evidence of hyperplasia, or only microscopic hyperplasia without obvious enlargement of the glands. In the exceptional case, the parathyroids showed no evidence at all of hyperplasia. Nevertheless, this case, too, showed osseous changes sufficiently pronounced to justify placing it in the moderately advanced group. However, in no instance was there parathyroid enlargement of the degree present in the group mentioned [8-12]—the cases commonly classified under the head of "renal rickets" or its adult counterpart. The average weight of each parathyroid in those of our cases in which this weight was taken was 92 mg. per gland as compared with the normal average of approximately 30 mg. The most pronounced enlargement in this series was observed in a case of chronic glomerulonephritis of several years' duration with a history of an attack of acute nephritis 20 years previously. In this instance, the total weight of three parathyroid glands found was 467 mg., or, on the average, 156 mg. per gland. In accordance with the findings of Castleman and Mallory [7] in cases of secondary parathyroid hyperplasia, the enlargement of the parathyroid glands was due almost entirely to hyperplasia of the chief cells, the individual cells remaining of approximately normal size.

Discussion

The observations presented here on the development of secondary parathyroid hyperplasia in cases of chronic renal insufficiency

are in accord with previous reports. This hyperplasia may be due to the effect exerted upon the parathyroids by the hyperphosphatemia which is constantly present in progressive renal insufficiency,[17] although it can also plausibly be argued that hypocalcemia rather than hyperphosphatemia may be the instigating factor.[18] The pertinent question here is that of the possible relation of the parathyroid hyperplasia to the relatively mild bone changes we have noted in our cases of protracted renal insufficiency. In our opinion, these osseous changes are not altogether or necessarily ascribable to parathyroid hyperfunctioning. It may be said at once that there is as yet no undisputed method for the clinical estimation of parathyroid hyperfunction. Highman and Hamilton [19] in 1937 maintained that by the method of Hamilton and Schwartz they were able to demonstrate increased activity of the parathyroid glands in 20 of 23 cases of chronic renal disease. However, Gilligan, Volk and Gargill [20] were not able to confirm these results, finding the test to be negative 18 out of 19 times in 15 patients with marked chronic renal insufficiency, thus indicating, if the test is valid, an absence of hyperparathyroidism. In 4 of the cases with negative results, death occurred in uremia, 1 being a case of "renal rickets." All 4 showed distinct secondary hyperplasia of the parathyroids *post mortem*. On the other hand, interestingly enough, Gilligan, Volk and Gargill found the test to be positive in 3 of 8 cases of active Paget's disease of bone and in 7 of 18 cases of thyrotoxicosis.

To what degree, therefore, a parathyroid hyperfunction participates in the production of the osseous changes in cases of chronic renal insufficiency in which the parathyroids show but little gross enlargement or only microscopic hyperplasia remains a question. (One cannot, of course, argue that hyperplasia of an organ is *necessarily* an indication of increased function; it may represent merely a reactive response to a stimulus.) We have no doubt that in cases of "renal rickets" with pronounced parathyroid enlargement, and in those cases of chronic renal insufficiency in adults associated with advanced skeletal changes and pronounced enlargement of the parathyroids, parathyroid hyperfunctioning can play a secondary rôle in the evolution of the osseous changes. At any rate, in regard to those of our cases which showed only

microscopic or mild gross evidences of hyperplasia, we are not convinced that a secondary parathyroid hyperfunction necessarily underlies the osseous changes which we have described. Indeed, if it did, it would be difficult to correlate the tendency, emphasized by both Rutishauser and co-workers [13-16] and ourselves [21] to osteosclerosis, in these cases, with the known osteoporotic effect of parathyroid hyperfunction in human beings. It is true that one might oppose here certain experimental observations on the effects of protracted injection of parathormone in rats. One of us (H. L. J.[22]) has observed, as have others, that under these conditions an osteosclerosis may develop in rats. However, it is doubtful whether a valid comparison can be drawn between these experimental observations of osteosclerosis in rats and the osteosclerosis in adults with chronic renal insufficiency.

Albright, Drake and Sulkowitch [10] have even expressed doubt that the osseous changes in their case representing the adult counterpart of "renal rickets" were caused by hyperparathyroidism or hyperfunctioning of the hyperplastic parathyroids. They believed that metabolic study of their case indicated that excretion of phosphates in excess into the gastro-intestinal tract prevented absorption of calcium from the intestines and led to decalcification in the bones. Rutishauser has consistently emphasized the importance of acidosis in the production of these changes and has minimized the rôle of parathyroid hyperfunction in this connection. It is pertinent here to refer to the experiments of Jaffe, Bodansky, and Chandler,[23] who were able to produce a state of chronic acidosis in dogs placed on a diet containing ammonium chloride. When this treatment was associated with the additional factor of insufficient calcium intake in the diet, there resulted the development of osseous changes which exactly simulated the osseous changes of hyperparathyroidism. The parathyroids, in these animals, showed no hyperplastic changes.

For the present we are in full accord with Rutishauser's idea that chronic acidosis resulting directly from renal insufficiency plays the important rôle in the instigation and maintenance of the changes in question. We believe that because of the deficient ability of the inadequate kidney to form base, the organism seems forced to excrete fixed base in order to be able to eliminate the

acid end-products of metabolism. Among the fixed bases on which the organism draws are the calcium and magnesium stored in the bones, and hence the skeleton tends to become demineralized. With this abstraction of minerals, accompanied, as it probably is, by the exaggerating factor of deficient calcium absorption and utilization, reactive resorption and fibrosis of the skeleton is invited in turn. With periods of fluctuation of the renal insufficiency and acidosis, new-bone deposition occurs which, if it predominates to a great extent over the process of resorption, occasionally results in the occurrence of an unusual osteosclerosis.

Summary

In cases of chronic renal insufficiency in adults, there almost regularly occur skeletal changes consisting of more or less pronounced fibroporotic resorption of bone accompanied by a varying amount of new-bone formation. The latter may occasionally be so pronounced as to result in an actual osteosclerosis, observable grossly as well as microscopically. This appears to occur in those cases in which the state of renal insufficiency is long protracted and possibly fluctuates, so that phases of bone resorption and new-bone formation alternate but the latter predominates.

In these cases, there is also secondary hyperplasia of the parathyroid glands. While in exceptional instances this may be sufficiently accentuated to suggest that hyperfunctioning of these hyperplastic parathyroids has been a factor in the production of the osseous changes, in most cases the hyperplasia is only mild or moderate and there is no definite evidence to support the assumption of a parathyroid hyperfunction. We believe, therefore, that in most cases of chronic renal insufficiency in adults, the osseous changes commonly found are not due to "secondary" or "renal" hyperparathyroidism but are a consequence of the chronic acidosis induced by the renal insufficiency.

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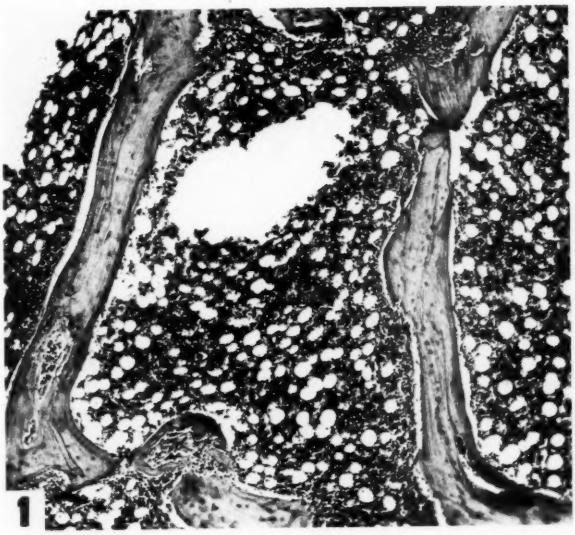
DESCRIPTION OF PLATES

PLATE 57

FIG. 1. Spongiosa of a vertebral body from a case of chronic renal insufficiency in an adult, showing relatively mild changes in the form of tracts of connective tissue containing osteoclasts in the haversian canals and on the margins of trabeculae. × 50.

FIG. 2. Spongiosa of a vertebra, showing moderately pronounced changes in the direction of osteosclerosis and already including some distortion and thickening of the trabeculae, due to apposition of new bone, in addition to the evidences of resorption. × 50.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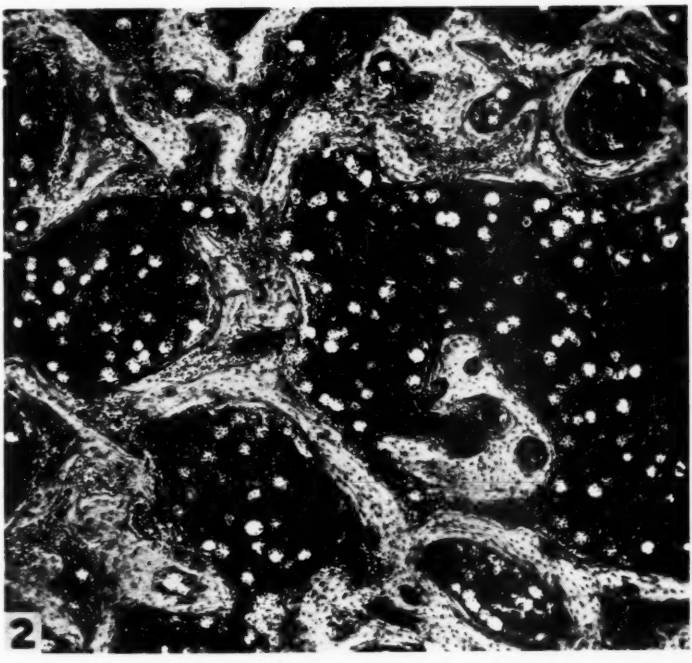
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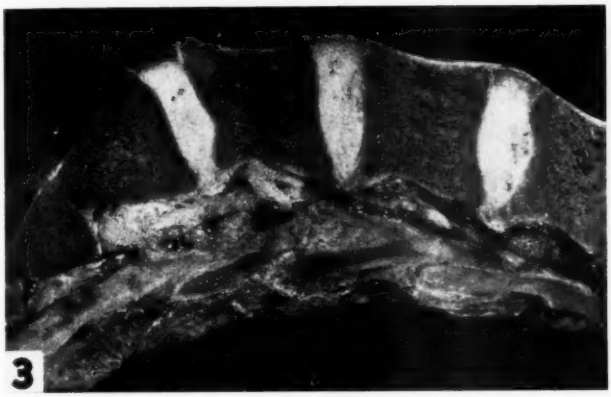
PLATE 58

FIG. 3. Photograph of the three lowest lumbar vertebrae and the sacrum, in longitudinal section, to illustrate a "renal" osteosclerosis so pronounced as to be evident grossly.

FIG. 4. Low power photomicrograph of the sacrum illustrated in Figure 3 to show the closely compacted spongiosa. × 2¼.



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A HISTOCHEMICAL STUDY OF THE DISTRIBUTION OF ALKALINE PHOSPHATASE IN VARIOUS NORMAL AND NEOPLASTIC TISSUES*

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Phosphatases having an *in vitro* P_H optimum of about 9 have been found in kidney, intestine, bone, and in smaller amounts in other tissues and in serum.[1] They have been stated to be involved in the absorption of glucose through the intestinal wall and reabsorption of glucose in the tubules of the kidney (Lundsgaard [2-4]), and in osteogenesis (Robison [5] and Kay [6]). Quantitative analytical measurements for the estimation of serum phosphatase have been developed by A. Bodansky and Jaffe,[7] and changes in serum phosphatase level were found in rickets, osteogenic sarcoma, obstructive jaundice, hyperthyroidism, and in other conditions.[1] Phosphatases were shown to differ by O. Bodansky,[8] who observed that the activity of bone and kidney phosphatases was retarded by bile acids, whereas intestinal phosphatase was unaffected.

Recently the introduction of a histochemical method by Takamatsu [9] and independently by Gomori,[10] for demonstrating the presence of phosphatase in tissues, has made it possible to study the location of phosphatase in cells and its distribution in different tissues.

The present study was undertaken to determine the presence of phosphatase in various normal and malignant tissues, and especially its relation to bone formation in a transmissible osteogenic sarcoma of fowls.[11] The data on the distribution of phosphatase were accumulated before we were aware of the studies of Takamatsu.[9] The histochemical method has also been used to study the inhibiting effect of various substances on the action of phosphatase in tissue sections.

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Method

The procedure outlined by Gomori [10] and by Takamatsu [9] is based on the deposition of $Ca_3(PO_4)_2$ at the site of enzyme action, when a section of tissue is incubated with an organic phosphate ester in the presence of calcium ions. In order to insure optimum activity of the enzyme, constant conditions and maximum histological definition, the following procedure was used:

Tissues were fixed in 95 per cent alcohol and paraffin sections were mounted on slides. The paraffin was removed with xylol and the xylol with absolute alcohol. The sections were then dipped in a dilute collodion solution, allowed to dry, hardened in 90 per cent alcohol,[10] and washed with distilled water. Alcohol under these conditions does not affect the stability of this enzyme. The sections were then transferred to substrate solution and incubated at 37° C. for 2 hours. Control serial sections were placed in a dilute (0.1 per cent) calcium nitrate solution. Stock solutions of 3.2 per cent sodium-β-glycerol-phosphate, 2 per cent calcium nitrate, 10 per cent sodium barbital, and 0.1 molar magnesium sulfate, were prepared. The solution used was made up by diluting 6 cc. of sodium-β-glycerol-phosphate, 9 cc. $Ca(NO_3)_2$, 6 cc. sodium barbital, and 6 cc. $MgSO_4$ to 60 cc., to give a final solution which was 0.01 molar with respect to glycerol phosphate and magnesium sulfate and had a P_H of 9. After incubation, each section was placed in the solution with its control and both stained for calcium by von Kossa's method, which replaces the calcium phosphate by metallic silver and gives a brown color at the site of phosphatase action. The sections were washed in absolute alcohol to remove the collodion, stained with hematoxylin and counterstained with light green. In the finished section the silver deposit (indicative of phosphatase) is brown, the cell nuclei blue and the cytoplasm green (Figs. 1-6).

Sodium barbital was used as a buffer since preliminary experiments showed very marked reduction and variability in the intensity of enzyme action in unbuffered solution. Magnesium, used in the concentration found to be optimal by O. Bodansky,[12] produced a slight increase in the intensity of the phosphatase reaction.

The Distribution of Phosphatase in Normal Tissues

The histochemical reaction showed the presence of large amounts of phosphatase in osteogenic tissues, renal epithelium and epithelium of the small intestine; that is, in tissues which form bone or are concerned with glucose absorption or elimination. In addition, small or moderate amounts of phosphatase were present in the endothelium of the blood vessels of most organs,

in certain nerve cells, in nerve fibers, in cells of the pituitary and in a few other types of cells.

Bone. The femur of a stillborn baby and bones of young chicks and of a mouse embryo were studied. Adult human bone could not be investigated because the usual procedure of decalcification results in the destruction of the phosphatase, but extracting the calcium salts from bones of a stillborn baby with diammonium citrate does not interfere with the demonstration of phosphatase.* The characteristic osteoblasts and the spindle-shaped periosteal fibroblast-like cells, wherever present, gave strong phosphatase reactions. Particularly instructive was the study of the embryonal bones. In the mesenchyme of the tail (Figs. 7 and 8) and leg rudiments large amounts of phosphatase were found in cells, which did not differ in appearance from the usual non-bone-forming mesenchymal cells. Proliferating cartilage cells in the chicken femur showed conspicuous phosphatase reactions. Cartilage in adult human trachea was free from phosphatase. Large amounts of phosphatase were found in the mandible and tooth anlage of a mouse embryo (Fig. 3).

Bone Marrow. Normal blood-forming elements, precursors of erythrocytes, granulocytes and megakaryocytes, did not give the phosphatase reaction. Some endothelial cells contained a small and variable amount of phosphatase. There were occasionally fine granules, indicating phosphatase action, scattered about the fat vacuoles.

Kidney. Adult human, and adult and embryonal mouse, tissues were examined. The cells of the convoluted tubules contained large amounts of phosphatase (Figs. 1 and 2). Their brush border was most intensely stained. The distal convoluted tubules, loops of Henle and collecting tubules contained no phosphatase, or traces only. The epithelium of Bowman's capsule gave an intense reaction if cuboidal, but was unstained if flat. The epithelium covering the glomerular tufts was free from phosphatase and only traces of phosphatase were found in the glomeruli.

Salivary Gland. In the lumen of a salivary gland about the acini, small amounts of phosphatase were demonstrated.

Intestine. Adult human, adult chicken and embryonal mouse

* Citrates have been used by J. Salk to dissolve calcium phosphate precipitates on which viruses have been adsorbed. (Personal communication.)

tissues were studied. The epithelium of the small intestine gave an intense phosphatase reaction (Fig. 6), most conspicuous in the upper layers of epithelial cells, while the deeper ones contained only a small amount of phosphatase. Autolyzed and desquamating epithelial cells also gave a marked reaction. A large amount of phosphatase was found in the endothelial cells of the submucosa. Occasional fibroblast-like cells of the submucosa likewise gave the reaction, while the muscle cells were free from phosphatase.

The epithelial cells of the large intestine did not give the reaction except a few cells in close proximity to the lumen. There were amorphous masses containing phosphatase in the lumen of the gut and also a few desquamated intestinal epithelial cells which contained only traces of phosphatase. Endothelial cells of the submucosa and of the lymph follicles almost invariably contained phosphatase and a thin film of phosphatase was found in the serosal layer.

Stomach. Epithelium of human gastric mucosa and that of the mouse embryo did not give the reaction.

Muscle. Adult human, and adult and embryonal mouse tissues were examined. Neither the heart nor the voluntary and smooth muscles examined contained phosphatase but the endothelium of capillaries gave a strong reaction.

Lung. Human adult, and mouse adult and embryonal tissues were examined. The usual alveolar epithelium did not contain phosphatase, but cuboidal alveolar epithelium contained some of this enzyme. In one specimen there was much edema fluid in the alveoli and this gave a faint phosphatase reaction. The endothelium, particularly of the large vessels, was well marked by this reaction. The epithelium of the bronchi and of the trachea contained small amounts of phosphatase. The cartilage of adult human trachea was phosphatase-free. Calcifying tracheal cartilage from an old individual contained no phosphatase about the calcium deposits. Much phosphatase was found in the basement membrane of the bronchial epithelium of an adult mouse. A small amount was found in the lumens of mucous glands and at the margin of this glandular epithelium in the trachea.

Liver. Adult human, chicken and mouse livers, and an embryonal mouse liver were examined. The endothelium of the sinu-

soids and of other vessels gave a conspicuous phosphatase reaction. The entire capillary bed, as a rule, was mapped out by this reaction. The bile ducts and liver cells contained no phosphatase, or only traces, although occasionally fine granules were seen scattered throughout the section but not identified with any cell type. In human liver of a young adult the liver cells contained a large number of very fine granules.

In order to distinguish between endothelial and Kupffer's cells a chicken was given intravenous injections of fine carmine particles. The animal was killed, the liver and spleen were fixed in alcohol and tested for phosphatase by the technic described. In the sections thus prepared, Kupffer's cells were filled with carmine particles, but failed to give the phosphatase reaction; whereas the endothelial cells of the liver contained large amounts of phosphatase but were free from carmine (Fig. 4).

Spleen. Adult human, mouse and chicken spleens and the spleen of a mouse embryo were studied. The endothelium, as in other organs, was well marked by the phosphatase reaction but none of the other elements of the spleen gave the reaction. There was some phosphatase in fibroblast-like cells about the margins of the trabeculae. The spleens of the mice contained erythrogenic and myelogenic foci with megakaryocytes, but none of these cells was phosphatase-positive. In the chickens that received intravenous injections of carmine the histiocytes of the spleen were seen to contain large amounts of carmine and were free from phosphatase, whereas the endothelium gave a phosphatase reaction but was free from carmine.

Lymph Node. Endothelial cells of blood vessels contained much phosphatase, while endothelial cells of most lymph sinuses were free from phosphatase. Most lymphocytes were free from phosphatase, though occasional groups of lymphocytes contained small amounts. Also occasional endothelial cells of lymph sinuses contained traces of phosphatase. The occurrence of phosphatase in lymphocytes and endothelial cells of the lymph sinuses was variable, and, if present, the amount of enzyme was small.

Adrenal. In sections from a mouse embryo the capsule and some cells of the zona glomerulosa gave the phosphatase reaction; the remaining cells of the cortex and those of the medulla did not. In the adrenal of a human adult, cells of the zona reticularis con-

tained the largest amounts of phosphatase, which was present in smaller amounts in both the fascicular and glomerular layers. The relative intensity of staining reaction, however, varied in different fields. In another section, from a woman who died *post partum,* large amounts of phosphatase were found in cells of the zona reticularis, moderate amounts in cells of the zona fasciculata and small amounts in cells of the zona glomerulosa. Cells of the adrenal medulla were phosphatase-free.

Pituitary of the Human Adult. In a pituitary from a man 71 years of age, neurogenic cells of the posterior lobe, the capsule and capillaries contained much phosphatase, as did clumps of epithelial cells in the posterior lobe. In the anterior lobe, only the capillaries and occasional clumps of epithelial cells gave the reaction. The type of epithelial cells which gave the reaction has yet to be identified, but their presence in large numbers in the posterior lobe of the pituitary of an old man makes it probable that they are basophils.

Thyroid. Thyroid epithelium from a young adult did not contain phosphatase. The capillaries in the same section contained phosphatase.

Testis. In a section from a human adult, most spermatogenic cells contained a small amount and the basement membrane contained moderate amounts of phosphatase.

Prostate. In the prostate of a young adult 18 years of age, the epithelium was found to be free from phosphatase. Some muscle cells contained traces of phosphatase. In an adult 64 years of age, numerous epithelial cells of the prostatic acini contained slight or moderate amounts of phosphatase. The staining reaction of the cells lining the ducts was of the same intensity. There appeared to be less reaction in the hyperplastic than in the non-hyperplastic acini.

A phosphatase with an optimal P_H in the acid range, the so-called acid phosphatase, was present in large amounts in prostatic tissue. Attempts are being made to develop a staining technic for acid phosphatase characteristic of this organ.

Seminal Vesicles. In a section from a human adult the epithelium was found free from phosphatase; the endothelium contained much phosphatase and the muscle traces of it.

Uterus. The myometrium was free from phosphatase. The

epithelial cells of the endometrium contained moderate amounts of phosphatase.

Bladder. The epithelium of the renal pelvis and of the urinary bladder of a mouse embryo gave a strong phosphatase reaction.

Pancreas. The epithelial cells of the pancreas from a mouse embryo and from a human adult contained no phosphatase.

Breast. In sections from the breast of a young woman *post partum,* and from another in the puerperium, the epithelial cells gave a strong phosphatase reaction, as did those in a fibroadenoma that will be described. There was also a small amount of phosphatase about the basement membrane and in the spindle-shaped, loose connective tissue cells between the glands. The colostrum contained only traces of phosphatase.

Ovary. In a section of ovary taken *post partum,* a very large amount of phosphatase was found in the corpus luteum, while the epithelium of adjacent primordial follicles contained none. Spindle-shaped, theca lutein cells surrounding the epithelial cells of the corpus luteum contained considerable amounts of phosphatase. The intensity of the phosphatase staining decreased gradually peripherally and none was found in the spindle-shaped cells distant from the corpus luteum. Capillaries almost invariably contained phosphatase; germinal epithelium was free from it. In a follicular cyst, granulosa cells lying free in the lumen contained no phosphatase, whereas several layers of polygonal and spindle-shaped cells about the cyst contained very large amounts of phosphatase. In a corpus albicans no phosphatase was found.

Phosphatase in Tumor Cells

I. Chicken Tumors

Osteogenic Sarcoma. The sarcoma studied is readily transmissible in chickens and is probably produced by a filterable agent.[11] Numerous samples were examined, some showing osteoblasts with almost no intercellular substance, and others showing mature bone, cartilage, and various stages of bone and cartilage formation by the malignant osteoblasts (Figs. 15–18). These osteoblasts were large round cells, slightly larger than large lymphocytes, with a large vesicular nucleus and a scant amount of cytoplasm. If seen with no osseous or cartilaginous matrix, their

osteogenic character was not evident. The phosphatase tests showed, however, that they contained phosphatase in increasingly large quantities when cartilage or bone formation was taking place.

Fibrosarcoma. Cells of a transmissible fibrosarcoma (strain 11 [13]), produced by a filterable agent, contained no phosphatase. This enzyme was also absent in cells of sarcoma and malignant endothelioma produced by another filterable agent.[14] Transmissible sarcoma cells originating in a growth produced in the breast of a chicken by methylcholanthrene likewise failed to give the phosphatase reaction.

Fowl Leukosis. The leukemic cells in the liver, bone marrow and spleen of chickens injected with viruses of fowl leukosis (strains 1 and 2 [15]) contained no phosphatase.

II. Mouse Tumors

Cells of a transmissible mouse sarcoma derived from mice that had been injected with methylcholanthrene failed to give the phosphatase reaction.

Adenocarcinomata of mice arising spontaneously in stock C_3H were likewise phosphatase-negative.

A spindle cell sarcoma, the osteogenic character of which was at first not recognized in the usual hematoxylin and eosin preparation, was found to contain very large amounts of phosphatase (Figs. 19 and 20). A careful study of the control sections showed, however, deposits of calcium granules in several places among the spindle-shaped, fibroblast-like osteogenic cells.

III. Rat Tumors

A liver carcinoma produced by feeding a rat with butter yellow (dimethyl-amino-azobenzene) was examined. The cytoplasm of the tumor cells contained a fine dust of brown granules. The necrotic parts of the tumor contained amorphous masses with granules. Most liver cells were free from phosphatase. The basement membranes of some cells, however, contained small amounts of phosphatase. The endothelial cells between the newly formed ductlike structures contained large amounts of phosphatase, while those of the sinuses of the relatively normal liver tissue contained none or only traces.

IV. Human Tumors

Carcinoma of the Breast. Sections from 4 carcinomas of the human breast were examined. None contained phosphatase.

Fibro-adenoma. A section showing the characteristic appearance of a fibro-adenoma of the breast, contained brown granules in large amounts in the cells of both acini and ducts and particularly abundant about the basement membrane (Fig. 5).

Carcinoma of the Gastro-intestinal Tract. Sections from a carcinoma of the stomach and from 7 carcinomas of the large bowel were examined. The latter included a mucinous carcinoma and 3 well differentiated adenocarcinomas. Phosphatase was absent in the tumor cells but present in the endothelium and in occasional fibroblast-like cells of the stroma (Figs. 13 and 14).

In a tumor metastatic from the sigmoid colon to the liver, no phosphatase was found in the tumor cells, while the connective tissue and endothelial cells of the stroma contained large amounts.

Liposarcoma. The section was taken from a tumor on the thigh of a man. The tumor cells were free from phosphatase. The endothelial cells contained a moderate amount and scattered cells resembling histiocytes between the malignant cells contained a large amount of phosphatase.

Malignant Melanoma. Sections from two examples of metastatic melanoma were studied. One was in an axillary lymph node, with the primary site undetermined. The other, in the small intestine, was a metastasis from the vulva. Neither contained phosphatase.

Adamantinoma. In this tumor from the lower jaw of a man, the endothelial cells and many of the connective tissue stroma cells contained small amounts of phosphatase, while the tumor cells contained none.

Hypernephroma. The tumor cells of a hypernephroma of the kidney, surgically removed, were free from phosphatase, while the capillaries were well marked by brown granules indicative of this enzyme.

Wilms' Tumor. Most tumor cells contained moderate amounts of fine granules indicative of phosphatase, while the stroma contained moderate or large amounts. The quantity of phosphatase in the stroma was sufficient to produce enough granules to obscure

the cells and it was not determined whether the phosphatase was free in the stroma or was in the fibroblast-like cells.

Perineural Fibroblastoma. The tumor cells of a malignant perineural fibroblastoma involving the sacral plexus showed no phosphatase.

Human Leukemia. Malignant lymphoid cells infiltrating the liver, spleen and other organs in a case of acute lymphoid leukemia contained no phosphatase.

Discussion

The following observations indicate that the histochemical method of Takamatsu [9] and Gomori [10] for phosphatase is specific for this enzyme. When this method is followed, except for the presence of sodium-β-glycerol-phosphate, only tissues containing calcium are stained; no non-calcium-containing tissues give the von Kossa reaction. The histochemical reaction is most pronounced in those organs (kidney, intestine and bone) which have been shown by chemical determinations on aqueous extracts to contain large amounts of phosphatase.

By the use of this histochemical method, the presence of phosphatase in tissues and organs not previously known to contain the enzyme has been established, and its relation to cells clarified. Thus, in the kidney the phosphatase is contained in cells of the proximal convoluted tubules. The endothelium, the cells surrounding embryonal hair follicles (Figs. 9 and 10), the myelin sheath of nerves (Figs. 11 and 12), the spermatogenic cells of the testis, and cells of the adrenal cortex have been shown to contain phosphatase. It will now become of considerable interest to determine what rôle phosphatase plays in these tissues.

The occurrence of phosphatase in vascular endothelium, for example, is of considerable interest in relation to the origin of the serum phosphatase. Armstrong and Banting [16] observed that removal of the viscera did not affect the serum phosphatase level and concluded that most of the serum phosphatase comes from bone. The presence of phosphatase in vascular endothelium may indicate either that this tissue contributes to the serum phosphatase, or that endothelial cells selectively remove phosphatase from the blood.

The presence of glycine had an inhibiting effect on the activity

of phosphatase. Thus, 0.25 molar glycine added to the substrate solution completely inhibited the action of phosphatase in bone, intestine and endothelium and almost completely inhibited phosphatase in the kidney in tissue sections. This is in agreement with the findings of O. Bodansky,[12] who demonstrated that glycine in concentrations higher than 0.00625 molar inhibited the action of kidney, intestine and bone phosphatase. This observation furnishes additional evidence for the specificity of the histochemical method.

No histochemical studies have hitherto been made on bone phosphatase, owing to the difficulty of decalcifying bone under conditions which did not destroy the enzyme. By the use of a 10 per cent solution of diammonium citrate, it was possible to decalcify bone without affecting the phosphatase. Kidney phosphatase was shown to be unaffected if the fixed tissues were kept for a week in diammonium citrate solution. Bone decalcified in this manner, however, gave poorer preparations than are usually obtained by other methods of decalcification. Additional data on bone phosphatase were obtained from developing mouse embryos, which can be cut with or without decalcification.

The method enables a differentiation between endothelial cells, on the one hand, and Kupffer's cells, histiocytes or monocytes, on the other. In one experiment Kupffer's cells and histiocytes of the spleen of a chick were marked by intravenous injection of carmine particles. Sections of liver and spleen showed no phosphatase in the carmine-laden cells, but the endothelial cells gave a good reaction (Fig. 4).

Lundsgaard [2-4] observed that phlorhizin, in addition to producing glycosuria, inhibits the phosphorylation of glucose, as well as the absorption of glucose, from an intestinal loop. These observations led him to postulate that glucose absorption in the intestine and its reabsorption in the kidney tubule are associated with intermediate phosphorylation, and that the phosphatase subsequently dephosphorylates the glucose phosphate. Lundsgaard subsequently became more cautious about this hypothesis with respect to reabsorption of glucose in the kidney because the dose of phlorhizin which completely inhibited glucose reabsorption per gram of kidney tissue was less (about one-fifth) than that necessary to prevent esterification in tissue. The histochemical

findings indicate that in the kidney the phosphatase is localized only in the proximal convoluted tubule,[9, 10] and if phlorhizin is also selectively localized in the proximal convoluted tubules its concentration might be high enough to inhibit the phosphorylation. Lundsgaard has himself observed a selective concentration of phlorhizin in the cortex of the kidney, and Ellinger and Lambrechts [17] demonstrated that phlorhizin dyes which produce glycosuria accumulate in the proximal convoluted tubules. The occurrence of large amounts of phosphatase in the lining epithelium of the small intestine also supports Lundsgaard's original hypothesis. None of the data presented by other investigators [18] directly contradicts Lundsgaard's original hypothesis.

Our results indicate that phosphatase occurs in cells of several different malignant tumors, the most important of which is osteogenic sarcoma. Malignant osteoblasts contain phosphatase even though there is no formation of cartilage or bone. This may be of some value in differentiating osteogenic from other tumors. For example, a sarcoma of the right arm, which was regarded as probable myosarcoma largely on the basis of its location, was found to contain large amounts of phosphatase. The malignant cells were fibroblast-like and because normal muscle cells are devoid of phosphatase it is unlikely that these malignant cells were derived from myoblasts.

Of four different strains of fowl tumors examined, only the cells of the osteogenic sarcoma contained phosphatase. Primitive blood cells from leukoses, like normal immature blood cells, were devoid of phosphatase.

Phosphatase was also found in a fibro-adenoma of the breast (Fig. 5), but not in four carcinomata of the breast. It is noteworthy that lactating breast also contains large amounts of phosphatase. These observations suggest that malignant transformation of mammary gland epithelium is accompanied by loss of this enzymatic function.

There are certain irregularities. Connective tissue, for example, is, as a rule, phosphatase-free. Occasionally, however, it contains small amounts of phosphatase. The fibrous connective tissue stroma of tumors often contained large amounts of phosphatase, even though the tumor cells contained none. These observations

suggest that young or proliferating cells contain more phosphatase than adult or resting cells. The phosphatase content of cells of the adrenal cortex varied greatly in different individuals and it is possible that this was due to changes in the functional state of the gland.

Muscle cells, as a rule, are phosphatase-free. Occasionally the sarcolemma takes the phosphatase stain, so that in cross section muscle fibers are surrounded by a bright brown honeycomb marking the sarcolemma sheaths.

Our observations with normal tissues are in essential agreement with those of Takamatsu[9] and of Gomori,[10] but there are minor discrepancies which will have to be cleared up by future work. For example, Takamatsu found that fibroblasts were free from phosphatase whereas in some of our sections these cells gave a definite phosphatase reaction. Takamatsu described strong phosphatase reactions in the epithelium of the large intestine, which were not seen in our preparations.

It is significant, as Takamatsu[9] noted, that endothelium of lymph vessels is phosphatase-free, while that of vascular endothelium contains phosphatase. This may be interpreted by assuming that the phosphatase of endothelium of blood vessels is derived from the plasma phosphatase which is absorbed by or deposited on the cells, or that the reaction is subtle enough to distinguish between endothelium of blood and of lymph vessels.

The observation that cells of a liver tumor induced in a rat by the feeding of butter yellow contained phosphatase was unexpected because liver and bile duct epithelium do not contain the enzyme.

It is noteworthy that embryonal precursors of phosphatase-containing adult cells also contain large amounts of phosphatase at the stage of embryonal development when there is no evidence of functional activity. Robison[5] has shown that mesenchyme of the mandibular anlage, incubated *in vitro* in tissue cultures, assumes histiogenic activity.

The histochemical reaction indicates that phosphatase is localized in the cytoplasm of cells. There is nothing known about its relation to other substances present in the cell. Recent observations[19] indicate that the enzyme is bound to a substance

sedimentable at high speed (approximately 27,000 r. p. m. for 1 hour), but upon autolysis the enzyme is liberated in an active, nonsedimentable form.

Summary

The technic described by Takamatsu and by Gomori for the histochemical demonstration of phosphatase is specific for this enzyme.

Alkaline-phosphatase activity is characteristic of certain cells. Among normal cells, the epithelium of the small intestine and of proximal convoluted tubules, osteoblasts, and endothelium are particularly rich in phosphatase.

Of the tumors studied, phosphatase is present in conspicuous amounts in the malignant osteoblasts of a transmissible chicken sarcoma, and in an osteogenic tumor of the mouse. In the osteogenic chicken sarcoma, phosphatase is particularly abundant about the sites of bony and cartilaginous deposits. In three non-bone-forming strains of transmissible chicken sarcoma, phosphatase is absent.

Human fibro-adenoma of the breast contains much phosphatase, as does lactating breast, while this enzyme is absent in carcinoma of the breast.

In the liver and spleen, endothelial cells alone contain phosphatase. Kupffer's cells and histiocytes do not contain phosphatase.

The presence of phosphatase in normal and malignant osteoblasts supports the view that this enzyme is important in bone formation and may aid in the histological identification of osteogenic cells.

The presence of large amounts of phosphatase in the intestinal epithelium is in accord with the views of Lundsgaard on the relation of glucose absorption to phosphorylation. Similarly, its presence in the proximal convoluted tubules of the kidney reopens the question of a similar mechanism explaining the reabsorption of the glucose secreted by the glomeruli.

Glycine, a known inhibitor of phosphatase action, inhibits also the phosphatase reaction in tissue sections.

Note: The authors thank Oscar Bodansky for many suggestions, and Fred Stewart for much of the pathological material used in these studies.

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DESCRIPTION OF PLATES

All sections were counterstained with hematoxylin and light green. The granules demonstrating the presence of phosphatase appear brown or black in the color photomicrographs.

PLATE 59

FIG. 1. Mouse kidney. × 600.

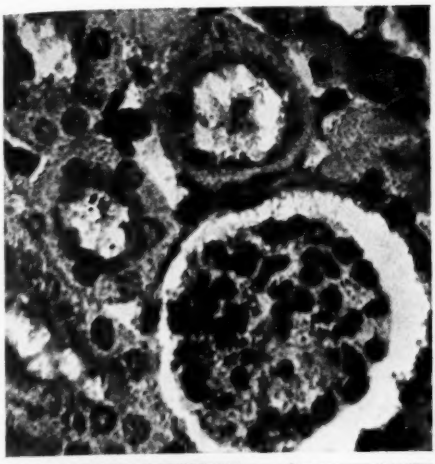
FIG. 2. Mouse kidney, control. × 600.

FIG. 3. Mandible and tooth anlage of a mouse embryo measuring 20 mm. in length. × 98.

FIG. 4. Chicken liver (carmine particles injected ante mortem). × 300.

FIG. 5. Fibro-adenoma of the breast (human). × 98.

FIG. 6. Small intestine of a mouse embryo. × 67.



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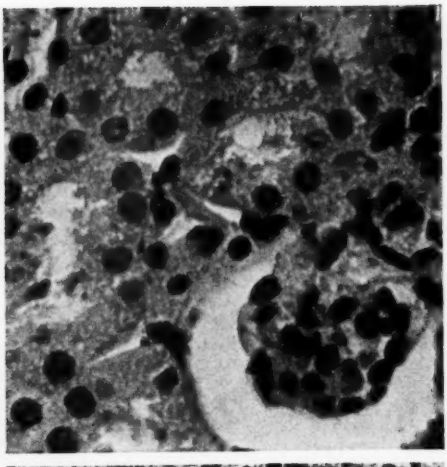

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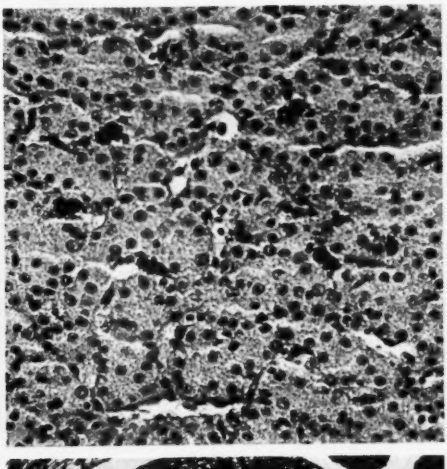

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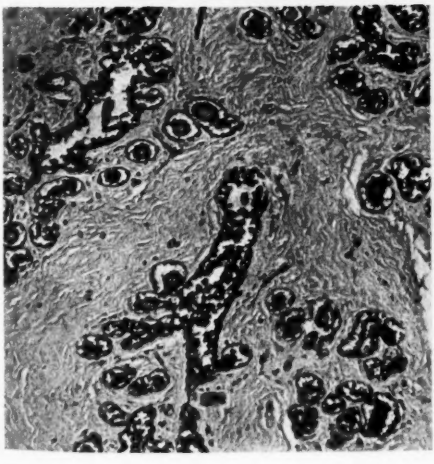

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Plate 60

Fig. 7. Osteogenic mesenchyme of the anlage of the tail of a mouse embryo measuring 20 mm. in length. × 150.

Fig. 8. Control section for that shown in Figure 7. × 150.

Fig. 9. Hair follicle of the scalp of a mouse embryo. × 180.

Fig. 10. Control section for that shown in Figure 9. × 180.

Fig. 11. Myelinated nerve fiber from chicken. × 350.

Fig. 12. Control section for that shown in Figure 11. × 350.

Fig. 13. Carcinoma of rectum (human). × 500.

Fig. 14. Control section for that shown in Figure 13. × 500.

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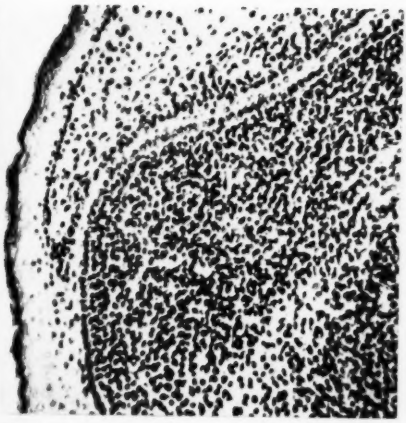

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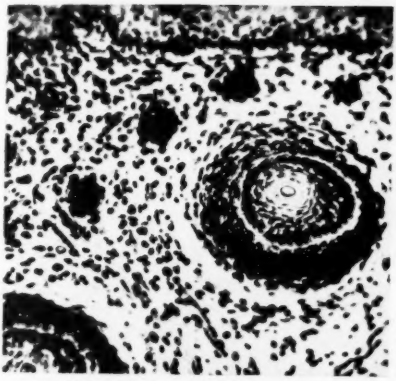

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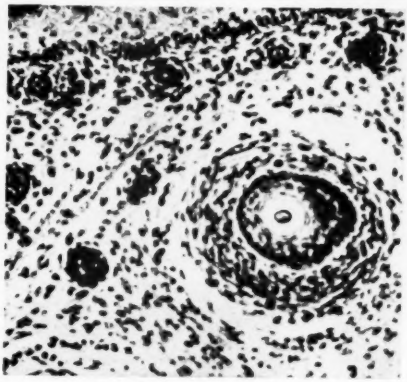

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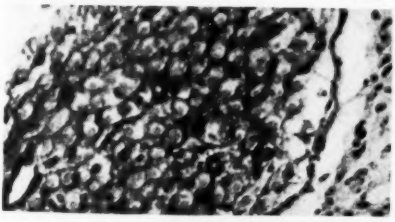

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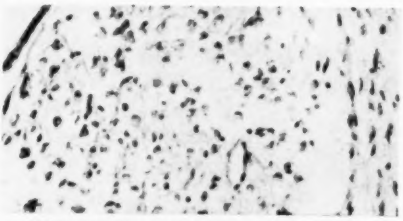

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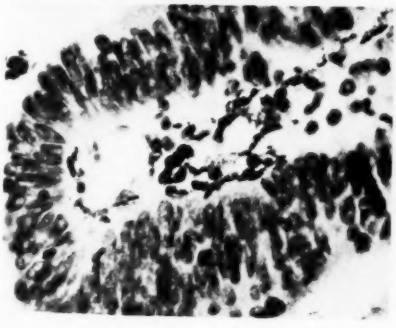

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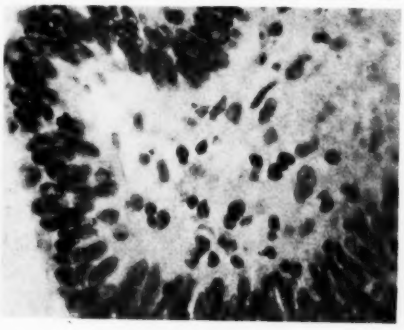

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PLATE 61

FIG. 15. Osteogenic chicken sarcoma. × 380.

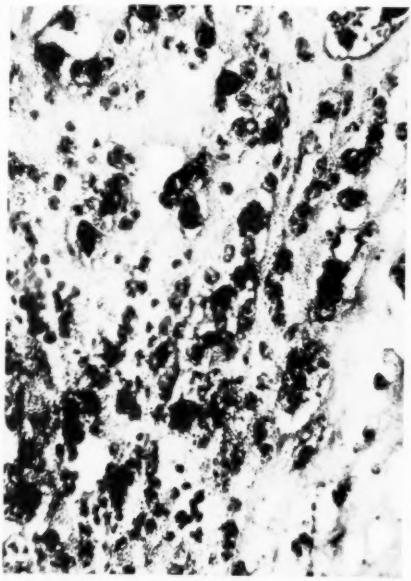
FIG. 16. Control section for that shown in Figure 15. × 300.

FIG. 17. Osteogenic chicken sarcoma. × 200.

FIG. 18. Control section for that shown in Figure 17. × 200.

FIG. 19. Osteogenic fibrosarcoma in a mouse. × 150.

FIG. 20. Control section for that shown in Figure 19. × 150.



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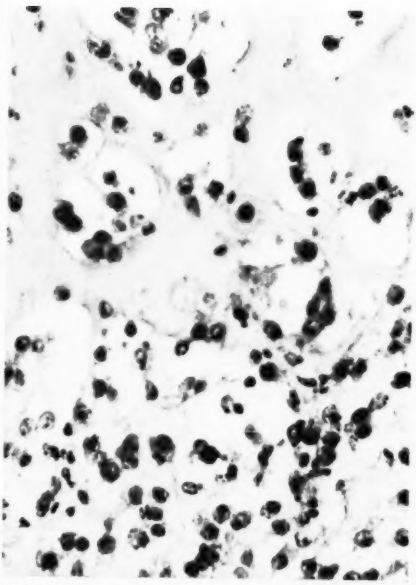

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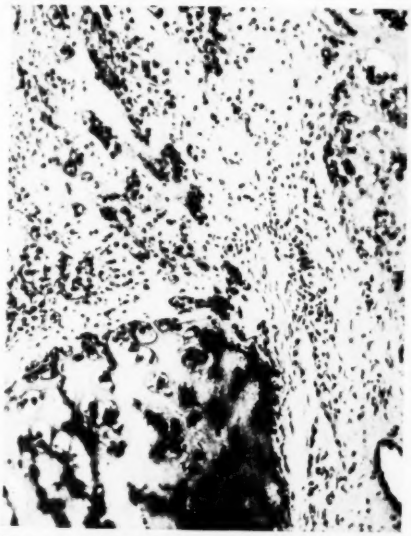

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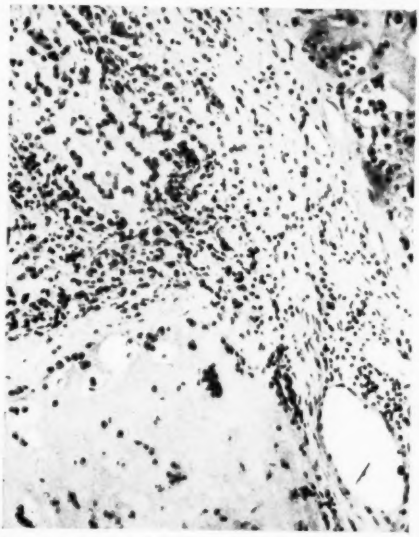

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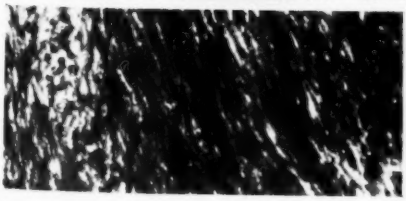

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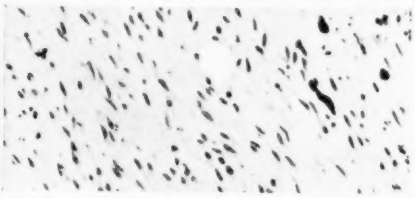

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COMPARATIVE EXPERIMENTAL STUDIES OF 200 KILOVOLT AND 1000 KILOVOLT ROENTGEN RAYS*

III. The Biologic Effect on the Skin of the Albino Rat

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In view of the growing interest evoked by million volt roentgen therapy, a series of experiments has been performed in an attempt to establish the histologic changes produced by this means of irradiation under controlled conditions; and to compare these changes with those caused by roentgen rays obtained from a 200 kv. apparatus. We have chosen the albino rat as an experimental subject without particular purpose save for the facility of handling these animals under laboratory conditions. It is, however, admittedly unreasonable to interpret results obtained in any lower animal in terms of expected effect upon man, and our conclusions are therefore offered with this point distinctly in mind. Certain experiments [1,2] have implied a greater degree of therapeutic efficiency of supervoltage (1000 kv.) rays than of high voltage (200 kv.) irradiation but clinical impressions have been somewhat varied.[3-6] Previous comparative studies performed by us have demonstrated that the histologic changes effected in the growing epiphysis [7] and the bone marrow [8] of the rat have been essentially similar with either form of irradiation. The present investigation is concerned with the lesions produced in the skin of the thigh in the same animal.

Methods

Since the procedure has been described in considerable detail in preceding reports,[7,8] only a brief outline will be given in this publication. Ninety-five albino rats of the same strain were obtained shortly after weaning and were sustained upon the Sherman "BMS" regimen.[9] At the time of application of irradiation

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they were 50 to 54 days old. All but 2 survived without evidence of intercurrent disease until such time as the needs of the experiment required their sacrifice.

The rats were divided into two major groups; group I received treatment with 200 kv. and group II with 1000 kv. roentgen rays. Each of these larger groups was further subdivided into six small subgroups, one of which (subgroup A) had no treatment and was retained for control purposes. Rats of the remaining subgroups were immobilized on a Y-shaped plywood board so that their hind extremities were abducted from each other and each rat was given a single dose of 600 r (subgroup B), 1200 r (subgroup C), 1800 r (subgroup D), 2400 r (subgroup E), or 3000 r (subgroup F), directed at the lateral aspect of the right thigh. In this manner 6 to 10 animals in each subgroup received equal doses from either machine. All dosages were measured in air with a Victoreen ionization chamber which was frequently checked with two others. The rats were then sacrificed by decapitation at prearranged intervals from 1 week to 19 weeks after irradiation.

The characteristics of the two machines utilized were as follows:

Group I Picker-Waite Machine		Group II Van der Graaf Generator
200,000 volts	Constant potential	1,000,000 volts
20 cm.	Distance	50 cm.
20 milliamp.	Current	0.85 milliamp.
0.25 mm. copper, 1.0 mm. aluminum	Filtration	3.0 mm. lead, 8.0 mm. copper
0.75 mm.	Copper half value layer	10.5 mm.
300 r/min.	Intensity	133 r/min.
10 cm.	Cone	10 cm.

Directly following sacrifice a strip of skin, including subcutaneous tissue and superficial muscle from the treated side of the thigh, was removed. Unfortunately, in the original experiments no material was excised from the medial aspect of the limb so that the effect of the "exit dose" could not be observed. The skin was immediately placed in Zenker's fluid with 5 per cent acetic acid and subsequently embedded in paraffin. Sections from all blocks were stained with phloxine-methylene blue. Con-

nective tissue and elastic tissue stains were applied when necessary for interpretation.

Observations

The material permitted histologic study of the effects of irradiation at graded intervals up to 5 months after the application of roentgen therapy. In addition it was possible to contrast the lesions produced by equal doses, as measured in roentgens, of rays developed by the high voltage and supervoltage machines.

Normal Rat Skin

The skin of the albino rat differs in several important respects from that of the human being. The epidermis in the region studied consisted usually of only two to four layers of squamous epithelium, over which a layer of partially desquamated keratinized material was loosely attached. Evidence of keratinization was present in the layer immediately overlying the basal columnar cells and the superficial layers were fully keratinized although they frequently contained stainable nuclei. Probably as the result of fixation, the surface was thrown into an irregular serrated outline (Fig. 1) but no recognizable epidermal papillae or rete pegs were present. The corium, except for a very narrow, finely fibrillar, subepidermal zone, consisted of irregularly distributed wavy bundles of collagen, among which a few thin-walled capillaries could be detected. The nethermost portion of the collagenous zone blended with a region composed predominantly of fatty tissue, in which were observed variable numbers of blood channels, still relatively thin-walled. This fatty zone was sharply demarcated on its deeper aspect by a thin layer of striated voluntary muscle. Penetrating the corium to a point variously proximate to the muscle were innumerable slender hair follicles arranged in a parallel manner. The bases or bulbs of the follicles were all situated in the fatty portion of the corium. Follicular walls exhibited stratification not unlike that observed in human hair although the thickness of the individual layers was considerably less. Each follicle at a point about midway through the corium gave rise to one or more oil gland appendages. The sebaceous glands consisted of cells with foamy cytoplasm indistinguishable from those in human skin but they were much smaller

and exhibited little angulation with the plane of the hair shaft. No sweat glands were present on the lateral aspect of the thigh. Elastic tissue stains demonstrated a fine intermeshed network throughout the upper portion of the corium, the general direction of the coarser fibrils being parallel with the surface.

200 Kilovolt Roentgen Rays

A dose of 600 r produced no histologic alteration in the skin of the rat. With 1200 r, however, a few dilated and congested capillaries appeared in the upper layer of the corium at about the third week. Similar changes appeared on occasion in the controls and therefore interpretation with any degree of assurance is not warranted. Nevertheless, despite the absence of any associated reaction, the congestion was considered to be the counterpart of clinical erythema.

During the first 2 weeks following doses of 1800 to 3000 r a more significant reaction appeared. There was slight swelling and vacuolization of the basal columnar layer and an accompanying increase in keratosis of the epidermis. Capillary congestion was apparent throughout the corium and there was an infiltration with lymphocytes, plasma cells and eosinophils arranged in a sprinkled fashion without characteristic distribution. Hair follicles exhibited slight swelling and vacuolization of sheath epithelium without remarkable variation in nuclear appearance. There was no evidence of epilation at this time. Subcutaneous blood vessels, fat, muscle and sebaceous glands were not appreciably affected.

Following the initial minimal reaction there was very rapid progression of events so that within the next 2 weeks maximal effects were apparent. There was complete epilation and, in the two subgroups receiving the highest dosage, complete epithelial denudation with ulceration and an intense inflammatory exudation in both corium and subcutaneous tissue (Fig. 2). At the edges of the eroded areas the surface epithelium was distinctly abnormal. Instead of two to four layers of epithelium there was marked thickening with as many as eight to twelve layers of cells. Deeper squamous cells exhibited vacuolization, hyperchromatism and, frequently, increased mitotic activity. In the upper three fourths of the epidermis there was an abundant

deposit of keratohyalin but even within the most superficial strata, nuclei were regularly visible. The inverted cuplike region of the hair follicle base disappeared and the lowermost portion of the persistent follicle became blunt and truncated. Sebaceous glands had disappeared at this stage and unfortunately none of our sections showed any intermediary phases of disintegration. Representing specialized follicular appendages, their existence was intimately related with the functional status of the follicle.

In this phase, marked congestion of blood vessels in all cutaneous layers was demonstrated, particularly in the subepidermal zone. Although there was a moderate degree of endothelial swelling with obtrusion into the lumen, no proliferative activity was noted at this time. There was a perivascular accumulation of inflammatory elements similar to that noted elsewhere in the corium and occasionally thrombosis and mural necrosis. The corium and subcutaneous tissues were widely infiltrated by varied proportions of polymorphonuclears, eosinophils, monocytes and lymphoid elements, the latter usually preponderating. A similar but less well marked reaction was present in the deep panniculus and in this region fat cells were shrunken and finely vacuolated, and their nuclei had become vesicular. Often they formed clusters surrounded by vacuolated mononucleated phagocytes and but rarely by multinucleated foreign body giant cells. The lesion represented incomplete fat necrosis.

Collagen bundles in the corium were separated by marked edema and were intrinsically swollen, fibrillar and much less wavy than usual. This was particularly evident in the more superficial region. Immediately subjacent to uneroded epithelium, accumulation of excess tissue fluid caused separation from the underlying corium, forming small fluid-filled, bleblike spaces. In the same region a few fibroblasts, divested of fibrillar matrix, lay free in the tissue spaces. These elements were swollen, stellate and contained abundant basophilic cytoplasm. Their nuclei were large, irregular and vesicular. Deeper in the corium the connective tissue, although not obviously affected, exhibited a homogeneous, dull, blocklike reaction to the various stains utilized. There was greater prominence of elastic tissue fibrils, which were also frequently split. In regions of more intense reaction and hair follicle destruction, the elastic tissue appeared as small clusters

of curlicue-like fragments. Their arrangement was usually perpendicular to or radiating from the surface plane instead of parallel, as is normally the case.

In general, following the fifth to sixth week after irradiation, the inflammatory reaction subsided and the denuded epithelium was regenerated. There were now, however, permanent epithelial alterations (Fig. 3). The epidermis was uniformly thickened, consisting of six to twelve layers of squamous cells, the superficial one half to one third of which were filled with dark-staining eleidin granules. Although somewhat hyperchromatic and pyknotic, stainable nuclei persisted within the most superficial cells. There was a moderately increased keratosis.[10] Hair follicle regeneration was scant, most of the follicles retaining the nonstratified stumplike appearance previously described. Such follicular remnants were rudimentary and had the appearance of exaggerated epithelial papillae (Fig. 4). The few follicles which were reëstablished were narrow and somewhat dwarfed in appearance. They contained normally arranged sheath layers and hair shafts, however, and small but well differentiated sebaceous glands were appended. Such restoration of hair as was grossly visible was sparse, fine and stunted.

In the corium the connective tissue exhibited a sharp division into two distinct layers. For a variable depth immediately subjacent to the epidermis there was diminished staining power and lessened collagenization, with increased prominence of fibrillae. In this region numerous thin-walled capillaries were evident, many of which had direct contiguity with the basement membrane of the surface epithelium. The latter was frequently separated from the underlying corium by narrow crevices, within which were observed innumerable tiny fibrillae forming fine sievelike fenestrations (Fig. 5). These maintained a degree of connection between the epidermis and cutis. Scattered throughout the superficial zone, frequently lying within the subepidermal crevices, were many large and elongated, stellate, basophilic fibroblasts.[11] Mitotic figures and multiple nuclei were occasionally noted in these elements.[10]

In the deeper stratum of the corium, collagen bundles were normally homogeneous but lacked the wavy, intertwining qualities apparent in untreated integument. The bundles were gen-

erally parallel to the surface plane in contradistinction to the irregular distribution evident in the normal state. A few swollen, atypical fibroblasts were present. Elastic tissue was seemingly markedly increased in amount and the fibrils were coarsened and irregular. In the region intermediary between the two zones of the corium there were numerous condensations of small, curled fragments but in the subepithelial regions scarcely any elastic tissue was found. In contrast with the normal fine meshwork of elastic fibrils arranged obliquely or parallel with the surface, the damaged skin manifested extreme irregularity and the direction of the fibrillar distribution was more commonly at an angle or perpendicular to the surface.

Blood vessels in the deeper cutis and subcutaneum were not remarkably increased in number or prominence. About many there was a considerable degree of perivascular fibrosis and a few retained a loose collar of lymphocytes and monocytes. Fibrils resembling elastic tissue (Gitterfasern) were unusually prominent in the media and even more so in the adventitia of some of the arteries.[12] Endothelial elements were not noticeably altered nor were vascular occlusive processes evident.

A marked grade of fibrosis was observed in both the subcutaneous tissue and the fatty panniculus. In the latter there was extensive lobulation as the result of the increased fibrous trabeculation. A peculiarity, probably an artefact but sufficiently constant to be worthy of note, appeared in these sections. In contradistinction to the irregular surface (Fig. 1) present in normal or minimally irradiated skin sections, the heavily irradiated specimens showed a relatively smooth surface which was only slightly, if at all, serrated (Fig. 3). This feature was undoubtedly the result of the effect of the fixative upon structures containing damaged connective tissue elements.[11,12]

1000 Kilovolt Roentgen Rays

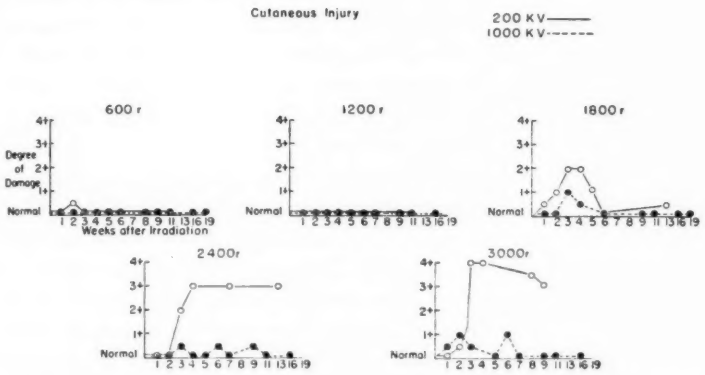
With dosages of irradiation up to 1200 r, the ultimate effects upon the skin did not differ significantly following either the high or supervoltage irradiation. Six hundred r from the 1000 kv. apparatus produced no recognizable changes whatever. With 1200 r superficial capillary congestion occurred, similar in all respects to that observed after the lower voltage rays, but de-

layed until about 1 to 2 weeks later. Higher doses, however, disclosed obvious variations in the effects caused by the two machines. The initial cutaneous response was essentially the same for both but again appeared 1 or 2 weeks later following supervoltage irradiation. This was in accordance with the clinical observations recorded by Stone.[5] Subsequent to the initial skin reaction, however, those animals receiving 1800 r or more, except for partial epilation, exhibited no further progression but exhibited instead a fairly prompt regression to a state indistinguishable from the normal (Fig. 6). In view of the albinism of the subjects, pigmentary stigmata were naturally not apparent. It was quite obvious that large amounts of roentgens (1800 to 3000 r), derived from 200 kv. radiations, caused profound destructive cutaneous alterations which, with subsidence, left permanent residua. On the contrary, equivalent doses of 1000 kv. rays caused, at most, insignificant changes which were promptly and permanently rectified. Animals exposed to supervoltage rays were observed for a considerably longer period than those in the high voltage group, so that it was evident that a delay in response could not explain the divergence in cutaneous reactions.

Discussion

The cutaneous reaction to roentgen rays is one of the most obvious biologic effects of these elements. For almost 4 decades it has been utilized to a greater or less degree as an index of radiation dosage. Since the scope of deep radiotherapy has been sharply limited by the intensity of skin injury, any means of circumventing this "barrier" has considerable clinical value. The experimental investigations of Failla [1,14] and the clinical observations of Dresser and co-workers [3,4] have suggested supervoltage rays as a means of avoiding much of this difficulty. Stone,[5,6] however, by clinical experimentation has concluded that no greater advantage appertains to supervoltage therapy than may be obtained with diligently controlled high voltage treatment. Since experimental observations upon the comparative effects of equal doses of the two types of rays upon bone marrow [8] and the epiphysis [7] showed no significant variations in the degree of alteration produced, the controversial question of relative skin sensitivity becomes an important one. Skin sections from our subjects have therefore been subjected to particularly detailed

study. Each structural component has been individually investigated in all stages of the reaction and conclusions drawn from the composite whole. Text-Figure 1 offers graphic comparison of the degree of damage effected by either form of irradiation. In contrast to the similar results observed in the growing epiphysis and bone marrow regardless of the voltage, doses measured in roentgens equivalent to those derived from a 200 kv. source, which cause severe and permanent skin changes, will, when obtained from the supervoltage apparatus, cause only insignificant cutaneous damage.



Text-Figure 1. Graphic comparison of the degree of cutaneous injury produced by equal amounts of roentgens of the two voltages used.

It has been suggested that calibration of dosage with the Victoreen dosimeter permits the entrance of a physical error which may account for the discrepancy in results.[13-16] According to this hypothesis a given dose of 1000 kv. rays measured in roentgens with the dosimeter would be equivalent to only approximately 75 per cent of an equal dose of 200 kv. rays determined by the same method. It is impossible to estimate the degree of biologic reaction obtained in this study in an accurate mathematical manner but it may be safely stated that the variation in the effect upon the skin was not 25 per cent, as anticipated by physical discrepancies, but as much as 200 to 300 per cent. It is believed, therefore, that the physical error inherent in dosimeter evaluation is inadequate to account for the degree of difference in reaction.

Some other cause for this variation must therefore be sought. The region treated, the age and metabolic status of the subjects, and the stock were all constant. Discrepancies could not be attributed to a difference in the biologic material utilized. Suspicion must then naturally fall upon the physical character of the roentgen rays applied. Failla [1] and Trump [17] have implied that the predominance of shorter wave lengths in the beam, developed by the supervoltage apparatus, produces maximum effects below the integument instead of at the point of surface impact, as occurs with the longer wave lengths produced at 200 kv. Our study has not included investigation of the tissues intermediate in depth between skin and bone and we are therefore unable to establish this point unequivocally. It is obvious, however, that the thickness of the irradiated parts was in all cases too small to make any diminution in the intensity of the primary beam an important factor.

We have not been able to confirm Stone's[5] clinical observations, from which he concluded that except for a delay in the appearance of erythema there was very little difference in the effect of the high and supervoltage radiation on the skin. Later observations [6] demonstrated not only similarities in minor initial reactions, a feature with which our observations have been in accord, but in severe delayed reactions as well. It is reasonable to suppose that the dissimilarity between Stone's experiences and those which we have had may be attributed to the nature of the material utilized. The rat's skin is unquestionably a very different structure from that of the human being and it is possible that its reaction to irradiation might differ widely. Other influential factors might arise from variation in the thickness of the part treated and in the density of its component structures. Since "backscatter" is of such significance in the development of tissue reactions and since this factor is closely related to the character and thickness of an irradiated part, it probably deserves major consideration in parallel comparisons of reactions in the rat and in the human being. Our material justifies interpretation only of the reactions observed in the rat. Comparative implications with the human being must be avoided.

In the rat, however, two mechanisms are possible. Either the

difference in the physical quality of the supervoltage rays finds the rat's skin relatively insensitive (wave length effect) or the hypothesis of a greater subsurface ionization effect must be invoked. The second of these two can be established by elimination. The question of wave length effect has been subjected to further experiment.

Twelve additional rats were divided into two groups of six. To each of these was administered a dosage of 3000 r (1000 kv.) in a manner similar to that already described. In one group, however, at the suggestion of J. G. Trump, the treated extremities were closely ensheathed to a depth of 0.5 cm. with a coating consisting of equal parts of beeswax and paraffin. This substance had previously been shown to possess physical qualities relative to roentgen rays not unlike those exhibited by mammalian tissues. Thus 6 animals received 3000 r directly to uncovered skin and 6 others were given the same amount of irradiation through a 0.5 cm. wax medium.

The purpose of this procedure was to determine whether the skin of the rat possessed inherent resistance to supervoltage rays, or whether these rays provoked tissue reaction beneath the surface layer without causing injury at the point of initial impact. Since the wax phantom caused the skin to become a subsurface structure, in accordance with our earlier experiments,[7,8] it might be expected to become as susceptible to 1000 kv. roentgen rays as the uncovered integument had been to 200 kv. rays.

Figures 7 and 8 exemplify the changes produced in both series at the peak of radiation reaction (4 weeks). It is quite obvious that the reaction produced in the unsheathed limb (Fig. 7) is relatively insignificant. Except for partial epilation on the lateral aspect of the thigh, no striking change is apparent. The paraffin-covered extremity (Fig. 8), on the other hand, exhibits not only complete epilation but in addition a profound cutaneous injury resulting in ulceration. Histologic modifications noted in this indirectly treated skin were quite similar to those already recorded following the administration of an equal number of roentgens from the 200 kv. machine.

The effect of the "exit dose" upon the skin of the medial aspect of the thigh, a feature neglected in the original experiments, was

investigated in the supplementary series. In those animals unshielded by paraffin it was found that the lesion appearing on this portion of the extremity was much more severe than that noted on the lateral aspect. The gross and histologic appearance, in fact, was exactly similar to that evident on the lateral side of the sheathed limb. There was complete epilation, permanent cutaneous damage and often widespread ulceration. The inner thigh of the wax-sheathed rats likewise showed more damage than the outer, and in some instances the severity of the lesion exceeded even that observed with the highest experimental doses given from the 200 kv. generator.

The observations of increased cutaneous damage as the result of artificial displacement of integument to a subsurface position by means of a wax phantom and the greater severity of the injury inflicted upon the medial aspect of the unsheathed extremities are complementary. They lend considerable support to the belief that variations in skin changes effected by high and supervoltage roentgen rays cannot be attributable to a wave length effect.

Summary and Conclusions

Equal amounts of roentgen rays, as measured with the Victoreen dosimeter, were obtained from a 200 kv. and a 1000 kv. generator. Two large groups of albino rats were given graded amounts of irradiation to the skin of the thigh from either of these sources. The character of the histologic changes produced at periods of 1 to 19 weeks after irradiation are described.

It was noted that high dosages of 200 kv. rays (1800 to 3000 r) caused severe and permanent injury to the skin and its appendages. Equal doses of supervoltage rays, however, caused only mild transient morphologic changes. The differences apparent in the lesions produced by the two types of rays are far greater than the amount of error attributable to the Victoreen dosimeter. The hypothesis is therefore advanced that the discrepancy must then result from either a specific cutaneous tolerance for supervoltage rays (wave length effect) or an enhanced skin penetrability, inherent upon the physical qualities of the rays themselves, due fundamentally to the difference in the

degrees of ionization produced by the two types of rays at the level of the skin.

By encasing the limb in a coating of beeswax and paraffin the skin was artificially transformed into a "subcutaneous" structure. It was then apparent that an amount of supervoltage rays which would cause only minimal damage to the skin under ordinary circumstances would, when applied through the medium of a layer of paraffin, produce a lesion similar to that found after like dosages of 200 kv. irradiation. It is believed, therefore, that the discrepancy in skin damage caused by the two types of rays cannot be the result of an inherent resistance of the epithelium to supervoltage rays.

It seems more reasonable to attribute this phenomenon to the fact that the ionization, to which the skin is subjected when irradiated by entering supervoltage rays, is different from the ionization which these rays produce some distance below the surface. Beneath the surface the ionization is probably more intense and contains relatively more low-energy secondary radiations. This is probably due to the fact that the "secondaries" produced by supervoltage rays are scattered predominantly in a forward direction so that a considerable thickness of tissue will be traversed before an equilibrium of primary and secondary ionization exists. In the case of the 200 kv. radiation this equilibrium is reached at a point much closer to the surface.

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DESCRIPTION OF PLATES

PLATE 62

FIG. 1. Normal rat skin. The thin epidermal layer is thrown into irregular serrations. There are numerous hair follicles, attached to each of which is a small sebaceous gland. × 60.

FIG. 2. Skin of a rat 19 days after irradiation with 3000 r (200 kv.). Surface epithelium is completely denuded and overlaid with a crust of partially necrotic exudate. Collagen is dull and blocklike in character and there is an intense infiltration with leukocytes. Hair follicles have been destroyed. × 60.

FIG. 3. Permanently damaged skin 47 days after treatment with 2400 r (200 kv.). There is marked thickening of the epidermis, which no longer exhibits the surface irregularity noted in the normal skin. Skin appendages are absent and there is dense blocklike thickening of the corium. × 60.

FIG. 4. Section from the edge of a heavily treated area (200 kv.) showing solidification of hair follicles with loss of normal stratification. Sebaceous glands have disappeared. The stumplike character of the residual follicles is evident. × 60.

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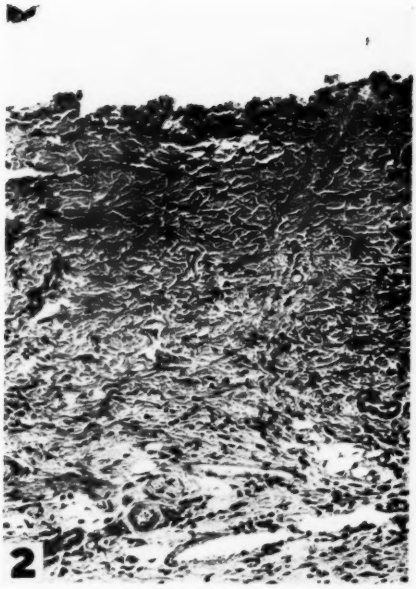
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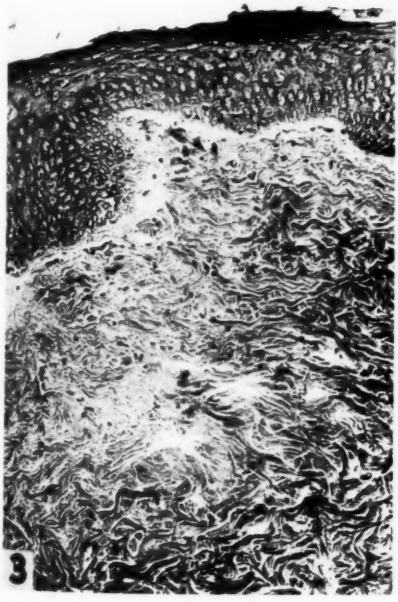
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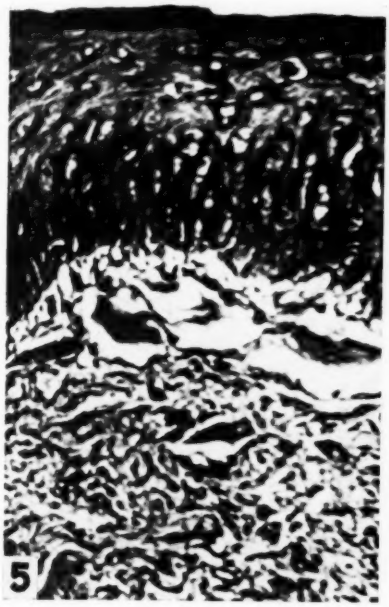
PLATE 63

FIG. 5. A high power view of the epidermal surface of the skin shown in Figure 3. The fenestrated, slitlike separation of superficial corium from epidermis is apparent. Within the corium are several large, bizarre fibroblasts. × 200.

FIG. 6. Skin section 21 days after the administration of 3000 r (1000 kv.). Except for a slight degree of keratosis the appearance is indistinguishable from that of untreated skin. × 60.

FIG. 7. The lateral aspect of the thigh of a rat 4 weeks after direct irradiation with 3000 r (1000 kv.). The cutaneous surface is intact and there is only partial epilation.

FIG. 8. The lateral aspect of a thigh 4 weeks after the administration of 3000 r (1000 kv.) through the medium of a 0.5 cm. layer of beeswax and paraffin. There is complete epilation, the skin is opaque and dull in appearance, and there is a large area of ulceration.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ALTERATIONS IN NUCLEAR VISCOSITY DURING EXPERIMENTAL CARCINOGENESIS DETERMINED BY ULTRACENTRIFUGATION*

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Experiments by Guyer and Claus (1939) seem to indicate that the long sought fundamental difference between malignant and normal cells may be one of viscosity. They write: "Judging from the lack of displacement of cellular contents in tumor cells subjected to centrifugalization at extremely high speed, the protoplasm of cancer cells, notably that of carcinomas and adenofibromas, has a decidedly greater viscosity than that of normal tissue cells." They explain that "since the nuclear contents are as little displaced by the centrifuging as are the cytoplasmic, there would seem to be a general stiffening up of the entire cell substance. The only other alternative would be the highly improbable supposition that all of the inclusions of the tumor cells have become of the same specific gravity as the general cytoplasmic ground substance."

The lack of displacements and the displacements observed by Guyer and Claus are well described and illustrated. We do not question them; but, without further evidence, we are not ready to accept their conclusions for the following reasons:

1. In order to subject the malignant cells to the same centrifugal force as normal cells, they implanted carcinomatous cells into the adrenal, kidney, pancreas and other organs of rats and, after growth had taken place, they centrifuged the malignant and the normal cells together. This ingenious method did not provide that direct comparison of malignant cells with their normal prototypes which is necessary to bring out the actual differences, because the malignant cells did not originate in these organs. Guyer and Claus reported that the resistance to displacement of the contents of tumor cells of various types is different. It is

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quite possible that the displacement in different normal cells may also be different. A false impression might be obtained if a tumor cell of a type highly resistant to displacement by centrifugal force were compared with a normal cell of characteristically low resistance.

2. Emphasis was placed by Guyer and Claus on differences between the displaceability of the contents of carcinomatous cells implanted into the adrenal and that of cortical adrenal cells, both of which were centrifuged together. Their Figure 15 shows no perceptible displacement of contents of the carcinomatous cells, while their Figure 14 demonstrates marked displacement of the contents of the cortical cells. Adrenal cortical cells are particularly rich in lipids, probably more so than the carcinomatous cells. Guyer and Claus employed an air driven ultracentrifuge of the Beams's type. They made no mention of a temperature change, but if an increase in temperature did take place in their experiments during centrifugation it would lower the viscosity of lipid-rich cells more than that of cells containing less lipid.

3. In general, one would not expect malignant cells, capable of multiplication and invasion and youthful in some respects, to be stiffer throughout their substance than normal cells of the same source. One would look, on the contrary, for greater fluidity and lower viscosity. Employing the technic of microdissection, Chambers and Ludford (1932) were unable to discover any differences in consistency among the cells of two mammary gland carcinomata, one tar carcinoma and two sarcomata and normal cells in tissue cultures. But, unfortunately, this comparison also lacked in directness.

The purpose of our experiments was to secure further data on the comparative viscosities of the nuclear contents of malignant and normal cells of the same type.

Material

This is indicated in Table I. The study of mice has been supplemented by the examination of human tissues.* The tissues of three strains of mice were employed.

1. New Buffalo mice constituted the main series. All, except

* Most of the human material was obtained from the clinics in the Barnard Hospital, but we are grateful to other St. Louis hospitals for additional specimens.

the embryos, newborn and normal adults, were treated with 0.6 per cent methylcholanthrene (Eastman Kodak Co.) in benzene. This was applied uniformly to the backs of the mice three times a week with a micropipette. Treatment was continued through-

TABLE I
Material

Tissue	Mice	Human
Adult epidermis	4, 8 and 12* months old	Female Negro, age 45; thigh Female Negro, age 48; abdomen Male, age 52; back
Hyperplastic epidermis	Treatment with methylcholanthrene for 11, 49, 51 and 64 days	From healing mammectomy wound in female, age 53
Papillomata	Treatment for 70 and 80 days	Warts from great toe of male, age 12, present 6 months; and from second finger of female, age 42, present 3 months
Squamous cell carcinomata	Treatment for 85, 99, 116, 130,* 140, 160* and 165* days	One each from helix of ear, posterior aspect of neck, cervical region, cheek, external auditory meatus, anterior auricular area, and two from lip
Transplantable tumors	One rapidly and one slowly growing MMS-21 sarcoma† Two rapidly and two slowly growing mammary carcinomas, 2163†	
Epidermis of newborn	12 hours old	Premature infant 7¾ months' gestation, lived 10 hours
Embryonic epidermis	18 days old	Two embryos of 12 weeks; one each of 16, 27, 31 and 40 weeks

* Roscoe Jackson Memorial Laboratory strain "A."
† Dobrovolskaia-Zavadskaia strain III.

out the periods mentioned, even after the appearance of grossly recognizable lesions.

2. A few strain "A" mice were treated in the same way.

3. Mice of the Dobrovolskaia-Zavadskaia strain III, bearing transplantable tumors also were used.

Methods

Like Guyer and Claus, we used an ultracentrifuge of the Beams's type. The internal diameter of our rotor was 2.3 cm. and its depth 0.55 cm. Whereas Guyer and Claus used compressed air as the driving force, we employed oxygen at a pressure of 60 lbs. per square inch. This gave us about 120,000 r. p. m.—a displacing force in the neighborhood of 350,000 times gravity. Guyer and Claus calculated their force to be 400,000 times gravity. We centrifuged for 30 minutes after attaining the speed mentioned while they centrifuged for 1 hour. In our experiments, as in theirs, the tissues were centrifuged in Locke's solution.

Conditions were standardized as well as possible. The rotor always felt distinctly cooler immediately after rotation by oxygen pressure than at the beginning. This decrease in temperature seemed to be uniform. In one instance room temperature was 26.8° C., and it was assumed that the Locke's solution in the rotor had become stabilized at the same figure. After centrifugation at the speed mentioned for 30 minutes, the temperature of the fluid in the rotor was taken by a thermocouple of constantan + copper wire, calibrated on a Leeds and Northrop type R galvanometer, using ice water and water at known temperatures from 8° to 25° C., and found to be 16.9° C.—a decrease of 9.9° C. When the ultracentrifuge was driven by compressed air at approximately the same speed for the same time, the temperature rose from 28.5° C. in the room to 32.0° C. in the Locke's solution in the rotor—an increase of 3.5° C. The decrease in temperature in our experiments in which compressed oxygen was uniformly employed would tend to increase viscosity and decrease the rate of autolytic changes.

The time intervening between excision of living tissue and its placement in the rotor was not always uniform. The transfer was made very quickly in the case of mouse tissues, the interval being probably less than 1 minute. For excised human tissues the interval was longer; but not sufficiently so, we think, to modify the results. Only in the case of human fetuses was the interval long enough to modify the results. On delivery, the fetuses were wrapped in gauze moistened with physiological salt solution and placed in an ice box at about 2.2° C. until they

were sent to us. Several were useless. We got the impression that resistance of nuclear contents to displacement increases with the degree of postmortem change. This was demonstrated experimentally as follows:

1. Two newborn rats were selected. Skin from the back of the first was immediately centrifuged while the second was wrapped in gauze, moistened with saline and kept in a closed glass vessel in an ice box at 1.2° C. for 20 hrs., after which skin was removed and centrifuged. Nuclei of spinous cells of both showed the same degree of displacement, but in the second the nuclei of the basal cells and of the roots of hair follicles in a few areas were not displaced as in the first and showed the typical postmortem appearance.

2. Three mouse embryos (age 18 days) were studied. The skin from 1 was centrifuged at once while the other 2 were kept in the same ice box for 36 hrs. The excised skin from the latter 2 responded differently: in 1 the displacement was similar to that in the fresh specimen and numerous mitotic figures were seen, while in the other some nuclei showed postmortem change and resistance to displacement. This difference suggests the operation of some other factor than length of time, possibly drying.

3. In a human fetus of 8 weeks, kept similarly in the ice box for 70 hrs., most of the epidermal cells were lost and the few that remained contained shrunken nuclei, the contents of which were not displaced.

It was found that perhaps the most important factor preventing displacement is drying of the tissue. If evaporation is permitted from the surface not protected by a keratinized layer, the nuclei of the superficial cells shrink, stain more homogeneously and appear to tighten up, for their contents become very resistant to displacement.

An effort was made to reduce all specimens to a uniform size and shape before placing them in the rotor. The dimensions were usually about 3 by 3 by 2 mm. The tissues were flattened a little against the periphery of the chamber of the rotor by the centrifugal force which decreases as the axis of the rotor is approached from the periphery. Calculations show that cells 1 mm. distant from the periphery (nearer the center) are subjected to a reduction of 4.34 per cent in force as compared with those at the periphery and that the force for the cells 2 mm. distant is 8.7 per cent less. The cells that we studied were almost invariably within the most peripheral millimeter.

The manner of orientation of the tissue in the rotor is important in some cases. In a piece of human skin, which was placed in the rotor with the dermis toward the periphery and

centrifuged, a peculiar condition was observed. Large cytoplasmic vacuoles appeared, chiefly in the spinous cells, rarely in the basal cells and never in the corneal cells. These vacuoles were characteristically spherical and usually there was but one per cell. They compressed the nuclei into crescent-shaped structures. The direction of nuclear compression was irregular and did not always correspond with that of the centrifugal force. In some portions of a given epidermis vacuoles and deformed nuclei were quite common, while in nearby parts they were absent.

When two samples of skin from the thigh of a normal white male 45 years old were placed, one with the dermis toward the periphery, the other reversed with the epidermis next the periphery, and centrifuged together, the vacuolation and resulting nuclear deformations were restricted to the first specimen. No explanation of this difference is offered. The phenomenon was never seen to the same degree in other tissues. It was occasionally manifested by a few cells in the epidermis of newborn mice. It was still less frequent in epidermis bordering papillomata and carcinomata but more often in human skin than in that of mice. Only in very rare cases was it observed in malignant cells of mice and of human tissues, accompanied by evidences of marked edema or of local necrosis.

For routine purposes a single histological technic was employed throughout. The fixative was Bouin's fluid. All paraffin blocks were cut in short strips of serial sections 5 μ thick. These were mounted and stained with hematoxylin and eosin. We intentionally used this relatively simple method because the results are fairly constant and the colors do not fade quickly, although a sharper contrast between basophilic (basi-) chromatin and acidophilic (oxy-) chromatin could have been secured by more selective staining.

To avoid misunderstanding it is desirable to characterize the terms we have used. Bodies in epidermal cells termed *nucleoli* usually stain with hematoxylin but they may possess a core of acidophilic material. They are more like amphinucleoli than true nucleoli or plasmosomes. They exist in living cells in approximately the same form and position. *Chromatin* is likewise stained with hematoxylin, often more deeply. It is seen as a cloud, in fairly discrete particles which grade in size up to false nucleoli

(net knots, basophilic nucleoli) and in amorphous masses, some of which cling to the nucleolus and to the nuclear membrane. We include under the same term, without any implication as to their nature, strands of material (reticulum, linin) which may be stained less strongly with hematoxylin or may even take a little eosin. The exact distribution of such chromatin in the living cell is not known, since it cannot be seen microscopically. It is safe to assume that part of the aggregation into masses is due to the action of the fixative. In considering its displacement, we must bear in mind that we are probably dealing with a fluid, partly made up of thymonucleic acid, which is present more diffusely in the nucleoplasm before centrifugation than when studied in stained preparations of normal cells. The *nucleoplasm* is often incorrectly referred to as nuclear sap because the word "sap" implies a fluid material like the sap of plants. This term should not be applied to a gel, which is sometimes the condition of the nucleoplasm.

Feulgen's reaction for thymonucleic acid was used in special cases according to the method described by Cowdry (1928). No preparations were made of the elusive cytoplasmic material, known as the Golgi apparatus, to which Guyer and Claus devoted considerable attention. Preparations were only made of mitochondria to gain information whether certain cells were degenerating.

Observations

The order of presentation is first to describe the displaceability of nucleoli and chromatin in normal epidermal cells and in our carcinogenic series of mice. Then an attempt is made to correlate displaceability with rapidity of growth of transplantable tumors. Finally the epidermis of newborn and embryonic mice is studied. The less adequate human material is described in substantially the same order. The tissues of mice (Figs. 1–16) and of human beings (Figs. 17–24) are briefly compared before the discussion of results.

TISSUES OF MICE

Normal Epidermis

After centrifugation (Fig. 1) there was no uniform displacement of nuclear contents. However, in small areas, especially

close to the hair follicles, the nucleoli were slightly displaced centrifugally. At the same time a shift of chromatin often occurred in the same direction. Mitotic figures were unchanged. There was no displacement of the contents of pyknotic and shrunken nuclei of the corneum. On the whole, the displacement was less in normal centrifuged epidermis than in any of the epidermal lesions of mice.

Hyperplastic Epidermis

Treatment with methylcholanthrene causes hyperplasia and thickening of the epidermis. In the normal epidermis of the mouse, one can distinguish only the inner cells and outer keratinized ones, while strata similar to those usually present in thick human epidermis were found in hyperplastic mouse epidermis.

After treatment for a period of 11 days only very slight signs of displacement of the nuclear contents of basal cells were noted. Many showed none. When present, displacement was more marked in basal cells near hair follicles. Chromosomes were not displaced. Nuclei of spinous cells are larger, more spherical, and less rich in chromatin than those of the basal cells. In them displacement was more marked (Fig. 2). Occasionally spinous cells were seen, the centrifugal cytoplasm of which stained more strongly than the centripetal cytoplasm, indicating, perhaps, a concentration of substance. In the corneum there was no displacement of the contents of pyknotic, shrunken nuclei.

Specimens centrifuged after treatment for 49, 51, and 64 days exhibited similar displacements of nuclear contents, though they were less marked. In some cells of the granular layer the nucleoli and chromatin were displaced while the cytoplasmic granules retained their positions unaltered (Fig. 3). In all specimens of hyperplastic epidermis the displacement was of patchy distribution. Areas were encountered in which none could be detected; but displacement was in general distinctly greater than in normal untreated epidermis.

From hyperplastic epidermis, a papilloma may develop which may or may not become malignant. From it, also, may develop squamous cell carcinoma.

Papillomata

The distal and better differentiated portion is made up of papillae which have grown outward. Figure 4 illustrates a section through a papilla cut in a plane vertical to the skin surface and parallel to its long axis. In the center is the vascularized core and on either side are basal cells which have given rise to spinous cells on the right and to keratinized cells on the left. Displacement of nuclear contents was very slight in the basal cells, more in the spinous cells and absent in the keratinized cells. Chromosomes were not displaced. This portion of the papilloma was obviously not in the line of carcinogenesis.

The proximal part, in which the cells are less well differentiated, consists of thickened and elongated processes which project into the dermis. A small area is represented in Figure 5. Comparison with Figure 4 shows that the cells and their nuclei are larger and the displacement of nuclear contents by centrifugal action was more marked. Other areas exhibit numerous mitoses and but little evidence of keratinization. In some places the chromosomes were shifted centrifugally. In several respects, therefore, the proximal parts of the papillomata are less differentiated than the hyperplastic epidermis which does keratinize, though the displacement of nuclear contents may be of about the same order—in some, a little more, and in others, a little less. The cells are not necessarily committed to become malignant, because even this proximal part of a papilloma does not always give rise to a carcinoma.

Squamous Cell Carcinomata

We deal with the origin of carcinoma from hyperplastic epidermal cells of the general skin surface but do not exclude the possibility that it may arise from the hyperplastic cells of hair follicles. The problem of the participation of hair follicles in carcinogenesis is left for consideration elsewhere.

Figure 6 shows, at low magnification, an area of hyperplastic epidermis on the left grading into a carcinomatous area on the right. Figure 7 illustrates, at higher magnification, a portion of the hyperplastic epidermis in which almost all the nuclei show displacement of nuclear contents. The tissue was treated with methylcholanthrene for 160 days.

It would appear to be a simple matter to trace the changes in degree of nuclear displacement when hyperplastic cells become malignant, but it is not so. There is no known microscopic evidence of malignancy observable in single cells by which its onset can definitely be dated (Cowdry, 1940). Malignancy can only be defined in terms of behavior and it is possible that cells may possess the power of invading other tissues before they actually break loose from their parent tissue and become able to exhibit it. However, since nucleoli and chromatin can be displaced by centrifugal force with considerable regularity both in hyperplastic epidermal cells and in the cells of a squamous cell carcinoma, their displaceability is not a sudden change which marks the assumption of behavior that we call malignant.

The degree of displacement is not uniform throughout a squamous cell carcinoma. None of the carcinomata studied in this series was large or of long standing. Consequently there was but little central necrosis. In all of them, however, there were a few scattered cells, often with acidophilic cytoplasm and small, or distorted and pyknotic, nuclei. These cells were evidently dead, or dying, at the time of fixation. Their nuclear contents were not displaced. Occasionally, mainly in low-grade carcinomata, groups of cells were seen which were rather small in size and fairly uniform in shape. Their nuclei contained finely granular, dust-like chromatin and one or two small nucleoli. In some cases, these cells made up the tips of processes extending into the dermis. In others, they were more deeply placed and separated from the epidermis. Their nuclear contents resisted displacement.

Displacement was greatest and most uniform in cells of variable size, shape, and staining reaction, characteristic of grade III or grade IV squamous cell carcinomata. Thus, displacement is seen in almost every active malignant cell included in Figures 8 and 9. The level of separation between the displaced chromatin and the clear nucleoplasm looks a little like the surface of a fluid. It is more definite than in either the hyperplastic spinous cells of the epidermis (Fig. 2) or in the hyperplastic cells of the proximal part of a papilloma (Fig. 5). These malignant cells, before centrifugation, were richer in chromatin than the non-malignant hyperplastic cells. This may have contributed to the formation of a more conspicuous separation level.

Figure 10 shows malignant cells fixed in the act of invading muscle. A large and a small muscle fiber extend from above downward and to the right. Both large and small nuclei have displaced contents, which are more evident in the large ones because there is more material to displace and a longer distance for it to move through.

In such active parts of a carcinoma, mitoses were quite numerous. The chromosome clumps were more uniformly and extensively displaced than in the cells of hyperplastic epidermis or papillomata (Figs. 11–13). In Figures 12 and 13, stainable cytoplasm is also represented as displaced. Evidence that the cells were active is given by mitochondrial preparations, for the mitochondria in nearby cells of the same general appearance were of the form, number, and distribution that one would expect to find in active, malignant cells.

TABLE II

Grade of Malignancy of Mouse Tumors and Percentage of Nuclei with Contents Displaced

Treatment	Grade	Percentage displaced
85 days, back	II	84.3
99 days, back	II	88.5
160 days, ear	IV	91.2
160 days, back	IV	97.1
165 days, back	III	98.0
165 days, back	II	91.9

Six squamous cell carcinomata were chosen for a preliminary quantitative study. In Table II, the duration of treatment with methylcholanthrene and the location of the tissue are given in the first column; the grade of malignancy, in the second; and the percentage of nuclei with displaced contents of 1000 examined in a typical area, in the last. Though much depends on the parts of the sections selected, and the numbers of nuclei examined were not large, there was a general correlation between grade of malignancy and percentage of displacement.

Transplantable Tumors

Efforts were made to discover the relation, if any, between the rapidity of growth of transplantable tumors and the per-

centage of their nuclei showing displacement of contents when subjected to equal centrifugal force.

Three pairs of mice* were used, with one in each pair bearing a rapidly growing tumor and the other a slowly growing example of the same tumor. The neoplasms were a sarcoma and mammary adenocarcinomata. Pieces of the rapidly and slowly growing tumors in each pair were centrifuged together.

At first sight, the results were unexpected and not in line with the observations on squamous cell carcinoma, for but little difference was noted in the incidence of nuclei with displaced contents in the rapidly and slowly growing sarcomata and mammary adenocarcinomata. Indeed it appeared that displacement was more evident in the slowly growing ones.

However, the comparison was not all that could be desired. Though the sarcoma grew rapidly in one mouse and slowly in its mate, it was not a case of two kinds of tumor cells possessed of different growth rates. On the contrary, the sarcomatous cells were of the same kind in both animals and therefore might be supposed to have nuclei the contents of which possessed similar displaceability under centrifugal force. How definitely fixed is this property in any type of malignant cell remains to be determined. It is possible that the difference in rate of growth of these sarcomata was conditioned by some difference in the hosts.

The same can be said for the carcinomata in the two pairs of mice. Again, it was a case of the same type of malignant cell growing rapidly in one mouse and slowly in its mate. This experiment does not, therefore, afford an answer to the question. We hope at a later date to compare the displaceability of nuclear contents of two different transplantable tumors, one endowed with rapid growth potential and the other with slow growth, subjected to equal centrifugal force.

Epidermis of Newborn Mice

The experiments were then extended to the epidermal cells of younger mice with the idea that these might in some respects resemble malignant cells more closely than the epidermal cells of adults already described.

* These animals were supplied by William H. Woglom of Columbia University.

Figures 14 and 15 illustrate the centrifuged epidermis of a newborn mouse. The first is from a region between hair follicles and should be compared with Figure 1 of the adult epidermis. The nuclei are not rich in chromatin, as in the adult, but displacement of nucleoli is more marked. In Figure 15, nearer to a hair follicle, there are more layers of nuclei which exhibit some displacement. The mouse had been born only 12 hours and the peripheral cells had obviously undergone quick desiccation and rapid keratinization. The keratinized layers were noticeably thicker than in the adult (Fig. 1). Mitoses were infrequent.

Embryonic Epidermis

In the epidermis of a mouse embryo of 18 days, which was centrifuged for only one half the regular time, the displacement of nuclear contents was very extensive (Fig. 16). The surface of this epidermis had not dried and the nuclei of the most superficial cells showed as marked displacement as the basal cells. The level of separation between displaced chromatin and clear nucleoplasm is even more definite than in malignant cells (Figs. 8 and 9). The uniformity of displacement, as of the size of the nuclei, is greater than in the carcinomata.

It is interesting to note that the shape of these embryonic nuclei was little, if at all, distorted by centrifugation. They were not drawn out into long strands with bulbous ends, like those observed in the tissues of embryonic chicks by Beams and King (1936) who used less force for a shorter time.

HUMAN TISSUES

Normal Epidermis

The nuclei of normal epidermis, as in mice, were very resistant to centrifugation (Fig. 17). In some places, especially near the tips of the rete pegs deep in the dermis, slight displacement of chromatin was detected in both basal and spinous cells. This, however, was not uniform. It was of patchy distribution and more often absent than present. In no case were chromosome clumps displaced. Nuclear displacement was not seen in the so-called clear cells near the basement membrane.

Hyperplastic Epidermis

In the epidermal cells at the edge of a healing wound, slight displacement of nuclear contents of about the same degree as in hyperplastic mouse epidermis followed centrifugation.

Displacement in hyperplastic epidermis is represented in Figure 18 from an area near a squamous cell carcinoma (Fig. 19). The contents of the nuclei of basal cells are but slightly displaced, if at all. In the spinous cells the shape of the nuclei is conspicuously changed. They look like crescents in clear vacuoles. These vacuoles are of approximately the same size as the spinous cell nuclei in uncentrifuged hyperplastic epidermis. It seems that, while the convexity of the centrifugal sides of the nuclei is maintained, the centripetal sides have been forced into the nuclear substance with loss of fluid from the nucleoplasm in a centripetal direction. From an original spherical shape the nuclei have become shallow cups and the volume of nucleoplasm has been sharply reduced. Some of the nuclei of spinous cells in hyperplastic mouse epidermis show similar but less extensive shrinkage on centrifugation. This is illustrated in Figure 2, which, however, was taken at a higher magnification than Figure 18. There is, therefore, a similarity in the effect of centrifugal force on human and mouse hyperplastic epidermis. This kind of nuclear deformation is not found in active carcinomatous cells.

Papillomata

Displacement of nuclear contents following centrifugation was slight and limited to the nuclei of a few spinous cells in a large wart removed from the big toe of a boy 12 years old; but no displacement of nuclear contents was observed in a smaller wart excised from the hand of an adult female. The resistance to displacement was a little greater than in the well differentiated, distal parts of papillomata of mice.

Squamous Cell Carcinomata

Figure 19 shows at low magnification the general character of the lesion from which Figures 20 and 21 were taken. A portion of an epithelial pearl is illustrated in Figure 20. The nuclei exhibit some displacement of nucleoli and chromatin. A nucleus below and to the left is shrunken and partially surrounded by

a large vacuole of approximately the same dimensions as was the nucleus before it lost fluid. The persistence of the original nuclear outline in the cytoplasm suggests that the cytoplasm is of firmer consistency. In parts of this carcinoma, where the cells are very actively invasive and show a marked nuclear hyperchromatism, there is an accompanying marked displacement of nuclear contents (Fig. 21).

Figure 22 is a photomicrograph at low magnification of a highly malignant squamous cell carcinoma. Hyperplastic epidermis is included below and to the right. Figures 23 and 24 are from the same tumor. The first shows the uniformity of nuclear displacement in hyperchromatic nuclei of a considerable range in size. As in the mouse tumor (Figs. 8 and 9), so also here the line of separation between chromatin and clear nucleoplasm is fairly even and implies a fluid level. The second (Fig. 24) is at a much greater magnification and shows that in certain nuclei strands of stainable substance stretch across the clear nucleoplasm in the direction of displacement of chromatin.

A quantitative study of squamous cell carcinomata was made by the same methods used for mouse tumors to show the percentage of nuclei exhibiting displacement. One thousand nuclei were examined in active areas where there were many mitotic figures. The results are presented in Table III, which should be compared with Table II for mice.

TABLE III

*Grade of Malignancy of Human Tumors and Percentage of Nuclei with Contents Displaced**

Location	Grade	Percentage displaced
1. Ear	III	89.3
2. Lip	II	88.5
3. Neck	II	85.5
4. Lip	II	81.2
5. Lip	II	70.4
6. Cheek	II	80.7

* A basal cell carcinoma showed 98.7 per cent of its nuclei with contents displaced.

Epidermis of the Newborn

Skin from a premature baby (7¾ months' gestation) that lived 10 hours was centrifuged in the usual way. The resulting specimen showed only very slight displacement in a small area

of spinous cells. The remainder of the epidermis had evidently suffered some desiccation.

Embryonic Epidermis

Pieces of skin from six fetuses (Table I) were centrifuged for 30 minutes. In all but one of them, however, the resulting specimens were useless because the tissue had been injured mechanically, allowed to autolyze or to dry. The single fetus that was of use for our purpose was of 4 months' gestation. It was dead when spontaneous abortion occurred and tissues were taken 55 minutes after delivery. Five pieces of skin from the back were immediately centrifuged for 30 minutes in Locke's solution. Five others were placed at the same time in Locke's solution for 30 minutes. Of these, two pieces were fixed in Bouin's fluid to serve as uncentrifuged controls and the remainder were centrifuged.

All of the centrifuged specimens showed considerable variability. In all of them some nuclei, usually in clumps, exhibited the shrunken, pyknotic appearance characteristic of both postmortem changes and drying. But in some there were fairly large stretches of epidermis in which the nuclei looked normal. In such regions two modifications were noted.

First, there was slight displacement of nucleoli and chromatin of epidermal cells and of the cells at the roots of hair follicles, but this displacement was of patchy distribution and many normal looking nuclei did not show it. In no case did the displacement even remotely approximate the stratification of nuclear contents in the embryonic cells of mice (Fig. 16).

The second and most consistent finding related to the position of nuclei in the epidermal cells. The direction of centrifugal force was from the interior toward the surface. Long rows of cells were observed with their nuclei located in the distal cytoplasm leaving the proximal cytoplasm clear. Nuclear shape, however, was not altered. In the uncentrifuged control specimen, a considerable number of nuclei were similarly placed but their distal position was in general less definite. Perhaps the centrifugal force enhanced a shift in position of the nuclei. Whether this peculiar location of epidermal cell nuclei is normal or results in some way from the many small injuries sustained by this tissue before examination, further work alone will show.

Discussion

Though we have found greater displacement of nuclear contents in malignant cells than in normal cells of the same sort, while Guyer and Claus observed less displacement in the tumor cells which they studied than in certain other normal cells, our findings are not incompatible with theirs because the tissues examined were different. Their observations relate to the entire cell substance and ours only to the nucleus. They assume that degree of displacement is conditioned by degree of viscosity and reject the possibility that a change in specific gravity of cellular components may be involved.

SPECIFIC GRAVITY

That substances of greater specific gravity are displaced more than those of lesser specific gravity is to be expected. Beams and King (1936) have reported the stratification of nuclear contents of embryonic chick cells "in the order of their decreasing specific gravity as follows: (1) nucleoli, (2) chromatin and reticulum and (3) nuclear sap." Ultracentrifugation has also been used to determine the weights of intranuclear inclusions caused by viruses relative to other nuclear components. Thus, Lucas and Herrmann (1935) have concluded that herpetic intranuclear inclusions are lighter than either the nuclear sap or the chromatin; while Rosenbusch and Lucas (1939) found that the intranuclear inclusions in the salivary glands of guinea pigs are heavier than the nuclear sap. But these observations are concerned with the relative displacement of nuclear components within the same nucleus. Our problem is to measure and explain the difference in displaceability between different nuclei.

We have been unable to discover any direct evidence either for or against a change in the specific gravity of nuclear materials in the transformation of normal cells into malignant ones. However, the nucleoli of malignant cells are sometimes slightly different both in size, number and appearance from those of their normal prototypes, and the amount of chromatin may be different. It is also possible that the water content of the nucleoplasm may not be the same. Cramer (1916) has reported, and it has since been confirmed, that the water content of various transplantable tumors varies directly with the rate of growth. Those

with most water grow the fastest. The same relation holds, to some extent, for normal tissues, since the tissues of embryos generally have a higher water content and grow faster than those of older individuals. Exceptions were enumerated by Needham (1931) and exceptions may likewise occur for malignant tissues. It is not known how the extracellular spaces, the cytoplasm and the nucleoplasm share in housing this relatively larger amount of water. Perhaps in both the malignant and embryonic cells which we have studied, the nuclei have a greater water content per unit of volume than those of normal adult cells, which might result in a lower specific gravity of their nucleoplasms.

Our finding of the greater displaceability of nucleoli and chromatin in malignant cells than in their normal prototypes might conceivably be due, therefore, either to an increase in the specific gravities of these components in the malignant cells as compared with normal ones or to a decrease in the specific gravity of the nucleoplasm, while the specific gravities of the components mentioned remained the same.

VISCOSITY

According to Heilbrunn (1937): "Viscosity can be roughly defined as the force which tends to hold the particles of a substance together when a shearing force acting on the substance tends to pull it apart." Viscosity, he says, is the inverse of fluidity.

To identify all the factors that may condition intranuclear viscosity is, of course, quite beyond us. Reference has been made to the likelihood of a change in the water content. But an alteration in the distribution of the same amount of water within a nucleus may also be important, as when a hydrosol gels. Utilizing the cessation of Brownian movement of particles in the nucleus viewed in the dark field as an indication of gelation, Lewis (1923) found that gelation of nuclear contents can be produced in living cells by weak acids and that return to the sol phase can be effected by washing with dilute alkalis, all without killing the cells.

It is probable that a swing of the reaction toward the acid side in nuclei of the dying or dead cells of those tissues, which in our experiments were intentionally kept for some time after excision

and before centrifuging, caused a gelation and so increased the density of their nuclear contents as to prevent displacement. Orr (1937) found evidence of increase in acidity during carcinogenesis in skin lesions of mice treated with dibenzanthracene and other substances by using phenol red as an indicator *in vivo*. If this acidity were to produce nuclear gelation, as the other acids did in the experiments of Lewis, we would expect an increase in intranuclear viscosity and a decrease in displaceability of nuclear contents under centrifugal force, which is the converse of what we have described. But the acidity may be of tissue fluids and may not extend to the cytoplasm or nucleoplasm. Results obtained by Rous (1925) and by Drury and Rous (1926) are compatible with this localization. Moreover, Chambers and Ludford (1932) injected indicators directly into the cytoplasm of malignant and normal cells in tissue cultures and could discover no significant difference in P_H.

While, therefore, admitting the possibility that a change in specific gravity of nuclear components may occur in the formation of malignant cells from normal ones and that this may partly condition the difference in displaceability, we think that it is safe to conclude that the main factor is a change in intranuclear viscosity and that in our series, easy displaceability of nuclear contents is a reliable indicator of low viscosity, and greater resistance to displacement an indicator of higher viscosity. In this qualified conclusion, we agree with Guyer and Claus.

Though practically all of the nuclei of the epidermis of the mouse embryo showed displacement, and almost all of the nuclei of active cells of squamous cell carcinoma also exhibited displacement, the phenomenon is less common in hyperplastic cells and almost negligible in the normal epidermis of adults. In the hyperplastic epidermis it is present in some areas and absent in others. Indeed, the nuclei of neighboring cells may differ markedly in their resistance to displacement. It may be that the intranuclear viscosity of normal cells of the same type and degree of differentiation is not constant, but varies with changes in physiological activity which do not find expression in structural changes detectable microscopically.

That differences in displacement of nuclear contents under equal centrifugal force are observed in cells unequally differen-

tiated, is not surprising. It is, however, interesting that the nuclei of spinous cells often show more displacement than the nuclei of nearby basal cells. This suggests a decrease in viscosity with differentiation of the spinous cells from basal ones. The nuclei of spinous cells are regularly larger and often more spherical than those of the basal cells. They contain less chromatin per unit of volume. Both of these differences are consistent with the assumption that their water content is greater. In their comparatively large nucleoli and small chromatin content they approach the nuclei of nerve cells and of ova, the contents of which are easily displaced.

It would seem that intranuclear viscosity, low in embryonic epidermal cells, rises in basal cells, falls in spinous cells, and rises again as the cells die because in the most superficial cells there is no displacement of nuclear contents. Similarly in the carcinogenic series it is low in embryonic epidermal cells, high in normal basal cells, lower in spinous cells and lower still in malignant cells, only to rise again as the malignant cells die.

Low intranuclear viscosity is not a characteristic of malignant cells which normal cells of the same type are unable to exhibit. But there are differences. The malignant cells which show it may be as rich in chromatin as embryonic cells of the same type, whereas the spinous cells contain relatively only a little chromatin. Moreover, the malignant cells may contain, proportionately to their size, as much chromatin as the basal cells of the epidermis in which the nuclear contents resist displacement. It is in the association of high chromatin content with low viscosity that the nuclei of malignant cells resemble those of the embryo. But in the descendants of malignant cells this property of low viscosity is fixed in so far that it reappears after each cell division, whereas it usually decreases during normal development.

We have made no attempt to measure the speed of these nuclear changes evidenced by groups of cells. The marked increase in viscosity between the embryonic and the newborn state of the epidermis of mice may be fairly abrupt owing perhaps to rapid desiccation of the superficial layer of cells. The slight decrease from normal epidermis through the higher grades of hyperplasia is probably much slower. The decrease in intranuclear viscosity with the formation of squamous cell carcinoma is very consider-

able and probably more than would be the case if the nuclei of the normal cells giving rise to the malignant ones had had an abnormally low intranuclear viscosity to begin with. Similarly, judging by ease of displacement of nuclear contents, the intranuclear viscosity of parenchymatous hepatic cells is relatively low. If the intranuclear viscosity of malignant cells formed from them is also low, the difference in intranuclear viscosities between this type of malignant cell and its normal prototype would not be very great. If, however, the intranuclear viscosity of this type of malignant cell happens to be high, the difference would be particularly noticeable because of the unusually low initial viscosity. It is, of course, the considerable decrease between the hyperplastic and the malignant cells, often with increase in chromatin content, that interests us most. Whether this is gradual or sudden, further work alone will show.

We have investigated only a single kind of malignant cell and its normal prototype. No generalization is justified. Data are awaited on the presence or absence of a similar decrease in nuclear viscosity in the transformation of other kinds of normal cells into malignant ones. When the skin, liver, kidney, pancreas, adrenal cortex and medulla of mice are subjected to equal centrifugal force, the displacement of nuclear contents is not uniform. It is very marked in the adrenal medulla, less in the adrenal cortex, kidney and liver and still less in the acini and islets of the pancreas and in the epidermis. Consequently it is important to note that in this study of epidermal cells and their malignant offspring, we commenced with normal cells, the nuclear contents of which are possessed of particularly high viscosity and are especially resistant to displacement.

Conclusions

When the nuclei in two pieces of tissue are subjected to equal centrifugal force, as a result of which nucleoli and chromatin are more displaced in one than in the other, there is reason to believe that intranuclear viscosity is lower in those in which the displacement is greater.

In methylcholanthrene carcinogenesis in mice, progressive changes in intranuclear viscosity occur. It is *high* in the nuclei of normal epidermal cells before the beginning of treatment,

lower in the nuclei of cells rendered hyperplastic in the treated epidermis and in the proximal parts of papillomata, and *much lower* in the nuclei of squamous cell carcinomata.

Passing backward from normal adult epidermis, through the epidermis of newborn mice to embryonic epidermis, there is also a decrease in intranuclear viscosity. In their relatively very low viscosity (lack of resistance to displacement of nuclear contents), the nuclei of embryonic epidermal cells closely resemble those of squamous cell carcinomata.

In a less complete series of human tissues, similar changes were observed. Intranuclear viscosity is *high* in normal epidermal cells, *lower* in hyperplastic cells of a healing wound and in papillomata (warts), and *much lower* in the nuclei of squamous cell carcinomata.

When, with degeneration, the nuclei of normal and malignant cells of both mice and humans shrink and become pyknotic, intranuclear viscosity increases so that it is impossible to displace their nucleoli and chromatin by the centrifugal force employed.

NOTE: We wish to express our appreciation to Burton Simpson and William S. Murray for supplying the New Buffalo mice used in these experiments; to William H. Woglom for the mice of the Dobrovolskaia-Zavadskaia strain III; and to Gordon H. Scott for the calculations. The strain "A" mice were obtained directly from the Roscoe Jackson Memorial Laboratory, Bar Harbor, Maine.

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DESCRIPTION OF PLATES

All of the photomicrographs are from sections stained with hematoxylin and eosin, prepared from tissues which had been centrifuged for 30 minutes, except Figure 15 for which the tissue was centrifuged for only 15 minutes. The directions of force are indicated by arrows.

PLATE 64

Specimens from Mice

FIG. 1. Normal epidermis. No displacement of nuclear contents. × 1040.

FIG. 2. Spinous cells of hyperplastic epidermis with marked displacement of nucleoli and chromatin of spinous cells. Treatment with methylcholanthrene for 11 days. × 1040.

FIG. 3. Cells of stratum granulosum of hyperplastic epidermis after treatment with methylcholanthrene for 49 days. The cell in the center shows displacement of nuclear contents without displacement of cytoplasmic granules. × 1040.

FIG. 4. Distal part of a benign papilloma after 60 days of treatment. The central core of vascularized dermis is bounded on either side by epidermis. Keratinization is more advanced on the left where there is no sign of displacement of nuclear contents. On the right, however, displacement is noticeable in a few large spinous cell nuclei and barely discernible in the smaller hyperchromatic nuclei of basal cells. × 1040.

FIG. 5. Proximal (deep) portion of a mouse papilloma which is less differentiated, showing displaceability in nuclear content of the cells. × 1040.

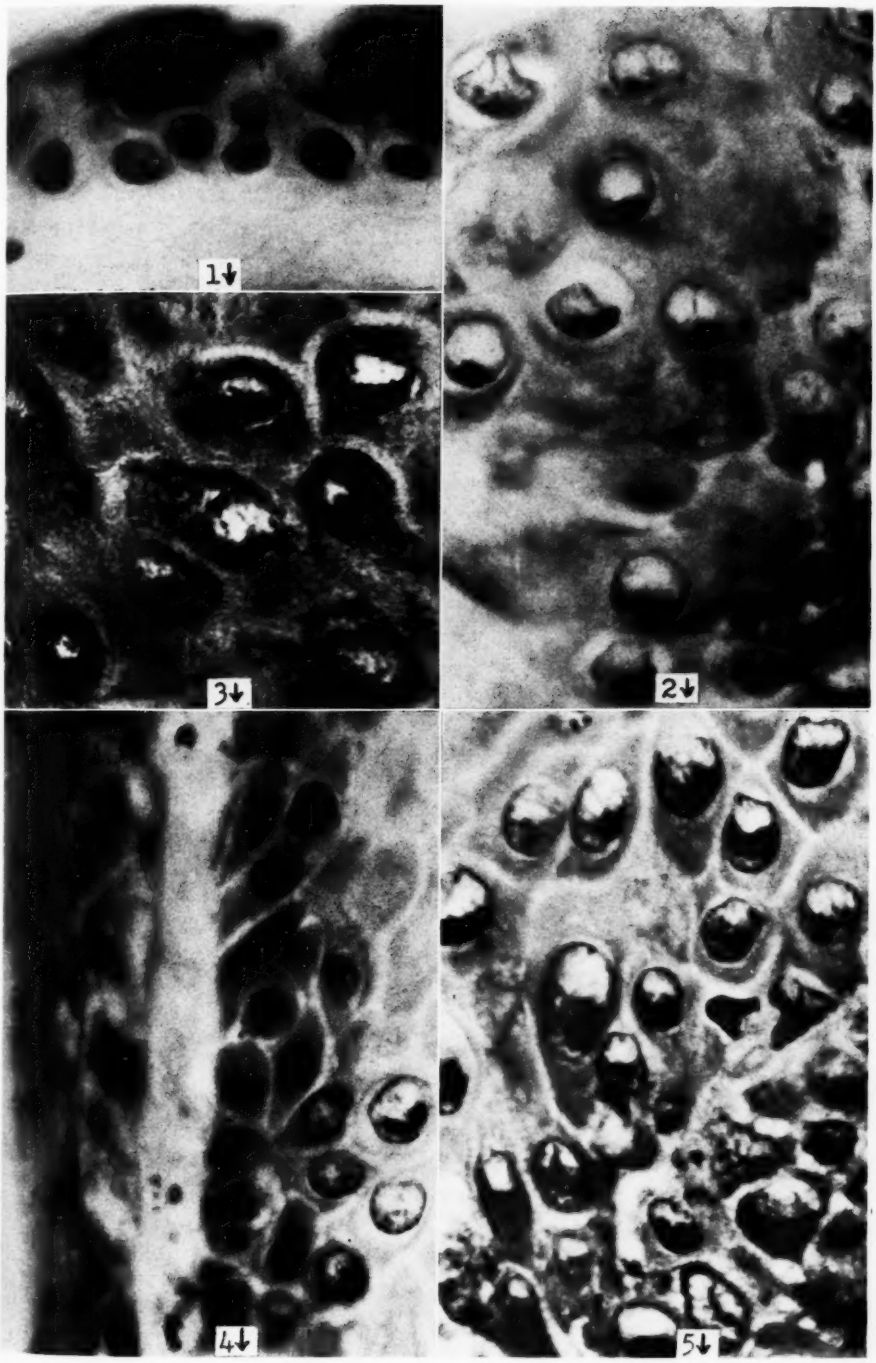
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PLATE 65

Specimens from Mice

FIG. 6. Hyperplastic epidermis on the left bordering on an area of squamous cell carcinoma. Treated with methylcholanthrene for 160 days. × 130.

FIG. 7. Higher magnification of hyperplastic epidermis in lower left corner of Figure 6, showing nearly uniform displacement of nuclear contents. × 800.

FIG. 8. Squamous cell carcinoma. Almost every nucleus of a malignant cell shows displacement of contents. Treatment for 116 days. × 1040.

FIG. 9. Squamous cell carcinoma with the nuclear contents displaced in large anaplastic cells. Treatment for 130 days. × 1040.

FIG. 10. Squamous cell carcinoma with malignant cells invading muscle. Their nuclear contents are displaced. Treatment for 140 days. × 1040.

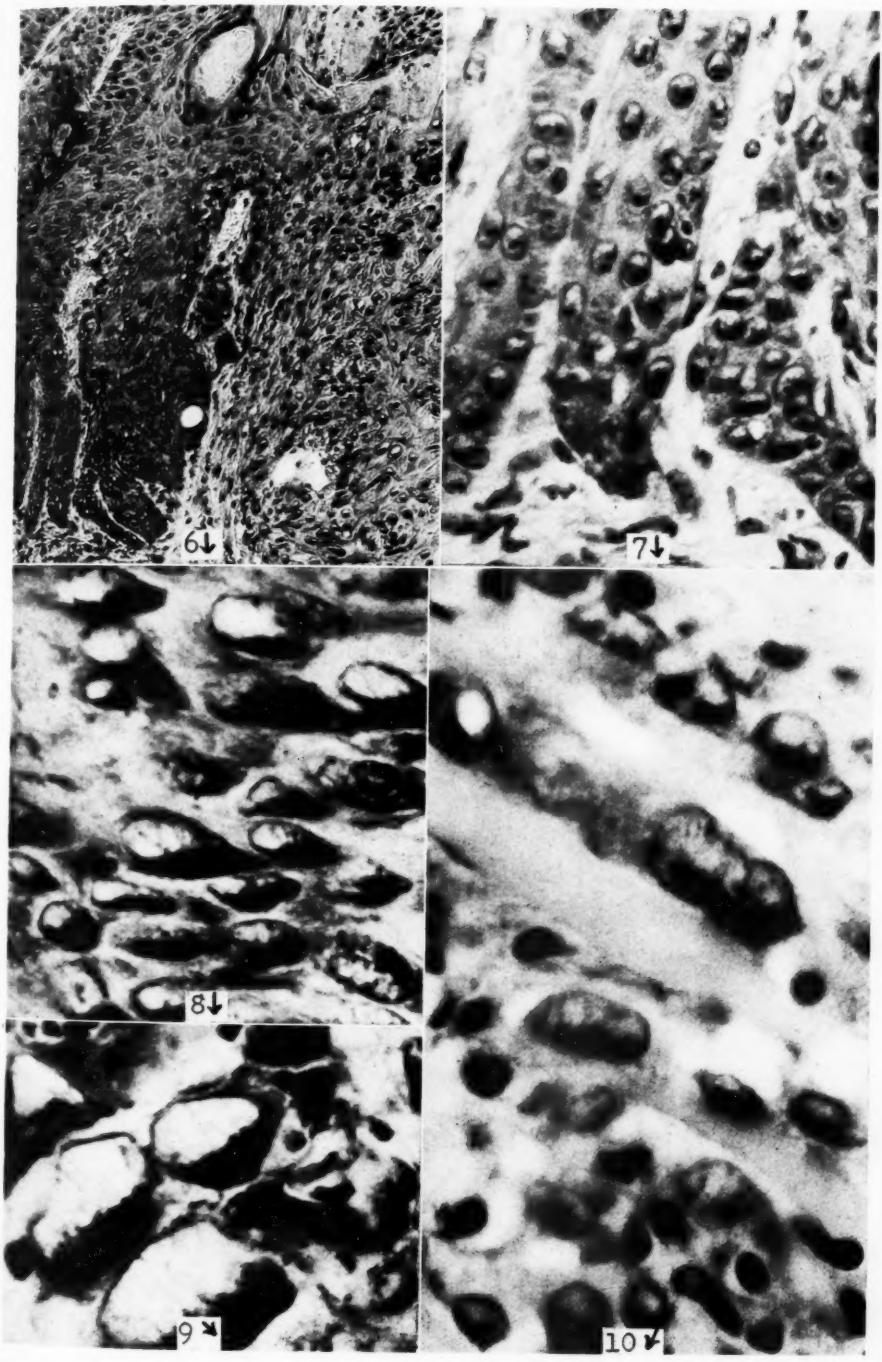
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PLATE 66

Specimens from Mice

FIGS. 11–13. Same specimen as Figure 6 but showing squamous cell carcinoma with dividing cells. There is displacement of chromosome clumps and the stainable part of the cytoplasm is also displaced in Figures 12 and 13. × 1040.

FIG. 14. The untreated epidermis of a mouse, 12 hours after birth. There is marked displacement of nucleoli and of some basophilic chromatin somewhat like that found in hyperplastic spinous cells (Fig. 2). × 1040.

FIG. 15. The same epidermis as in Figure 14, but near a hair follicle, also shows some displacement of nuclear contents. × 1040.

FIG. 16. Epidermis of an 18-day mouse embryo with extensive displacement of nuclear contents, even of surface cells. Compare with uniformity of displacement in squamous cell carcinoma (Figs. 8, 9, and 10). × 1040.

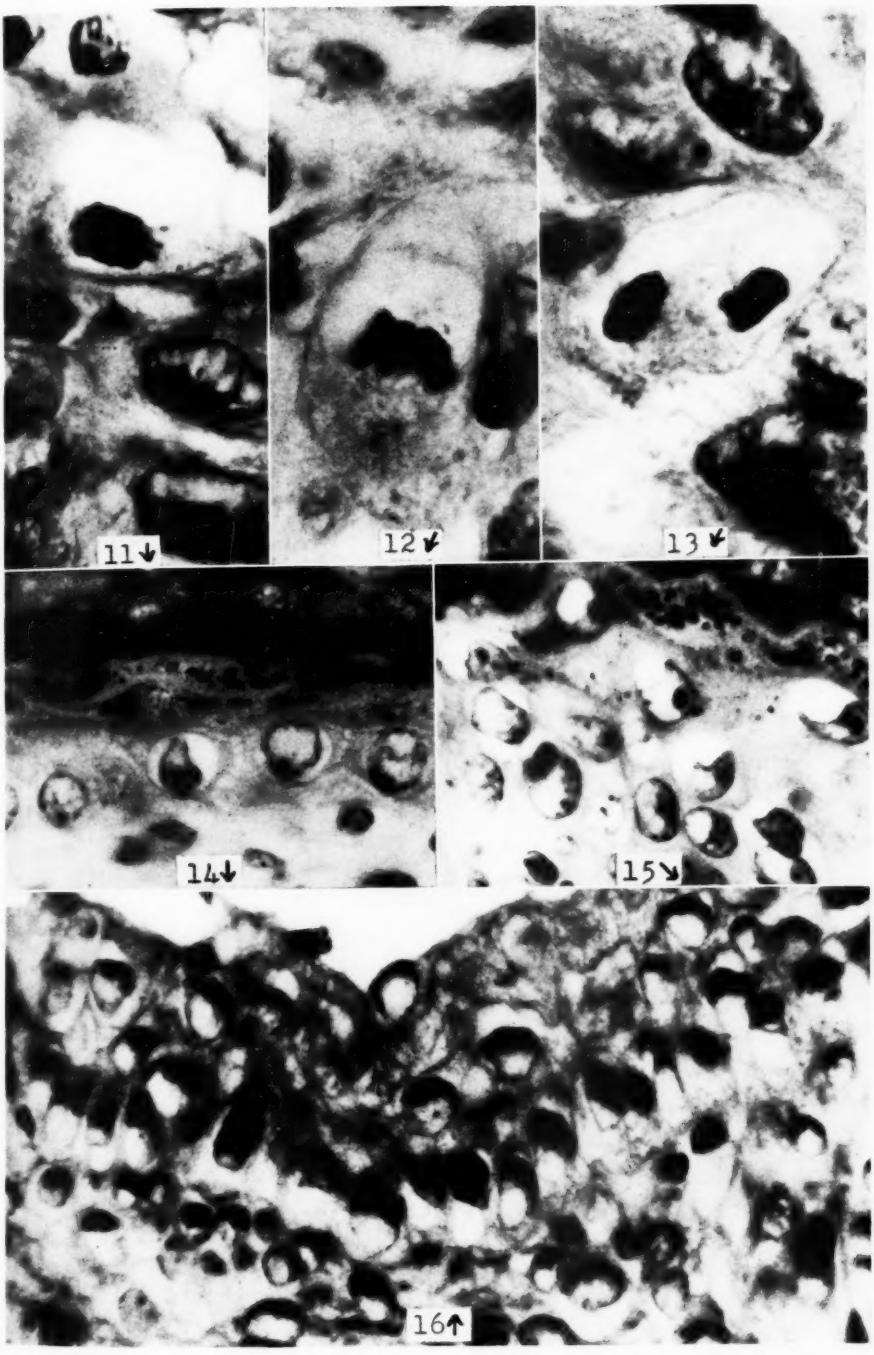
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PLATE 67

Human Tissues

FIG. 17. Normal epidermis from the back of a male 52 years old, showing very little sign of displacement. Compare with lack of displacement in normal mouse epidermis (Fig. 1). × 1040.

FIG. 18. Epidermis away from the transition point of the squamous cell carcinoma shown in Figure 19. There is very little displacement. × 700.

FIG. 19. Squamous cell carcinoma from the neck of a white male 58 years old, showing the character of the lesion. × 115.

FIG. 20. Displacement in the nuclei of an epithelial pearl. × 700.

FIG. 21. Considerable displacement in anaplastic cells invading deep tissues from the tumor shown in Figure 19. × 700.

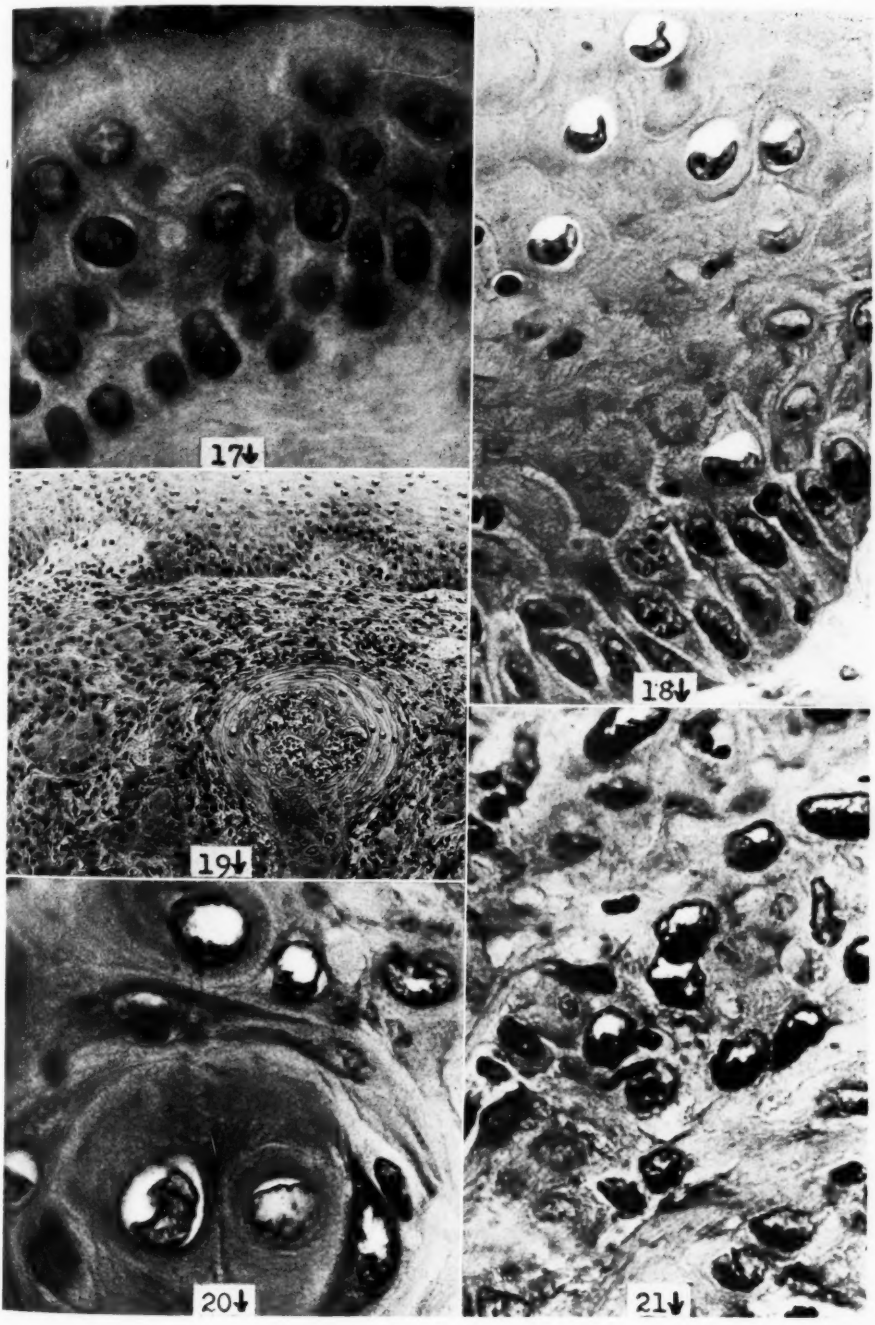
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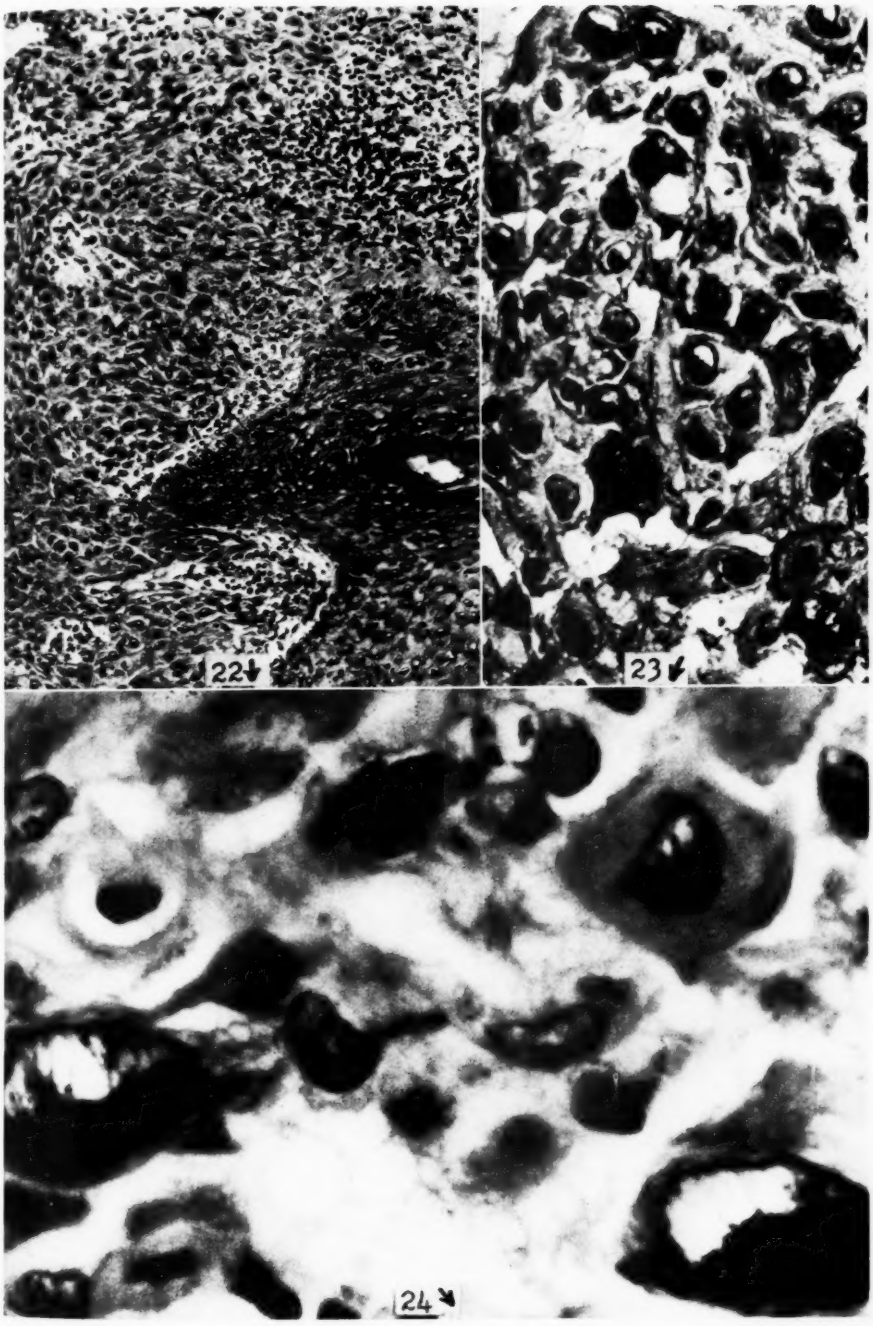
PLATE 68

Human Tissues

FIG. 22. Squamous cell carcinoma from an ear of a white male 54 years old, highly malignant. × 145.

FIG. 23. Very anaplastic cells from the same tumor as shown in Figure 22. Every cell shows displacement of basophilic chromatin. × 520.

FIG. 24. Higher power magnification showing displacement of basophilic chromatin in highly anaplastic cells of the squamous cell carcinoma illustrated in Figure 23. × 1500.



Cowdry and Paletta Alterations in Nuclear Viscosity

UNIV. OF MICH.

ACUTE NECROTIZING ARTERITIS, AORTITIS, AND AURICULITIS FOLLOWING URANIUM NITRATE INJURY IN DOGS WITH ALTERED PLASMA PROTEINS*

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In this paper are described the well marked necrotizing lesions which appeared in the aorta, sinuses of Valsalva, pulmonary artery, left auricle, and elsewhere in 5 dogs given uranium nitrate subcutaneously after being made hyperproteinemic by repeated intravenous injections of plasma obtained from healthy donor dogs. Similar lesions developed in 2 other dogs rendered hypoproteinemic by repeated plasmaphereses before injecting the heavy metal, and in still another dog which received only two injections of donor's plasma at a considerable interval before the injection of uranium nitrate. The factors common to all of these experiments are: (1) some alteration in the plasma proteins produced by the use of "homologous" blood and (2) uranium nitrate injury. While the nature of the process has not been revealed by the experiments thus far, the lesions are striking, can easily be recognized both grossly and histologically and have been produced in the relatively short period of about 6 weeks.

Necrotizing arteritis has attracted attention chiefly because of its occurrence in periarteritis nodosa and rheumatic arteritis. Numerous workers [1-4] have postulated a peculiar allergic or hyperergic state of the blood vessels to explain the extensive necrotizing lesions sometimes seen in these diseases. The following two quotations from the rather extensive literature have been selected as pertinent: Klemperer,[5] in discussing a case of periarteritis nodosa, stated: "I feel that we should not look for a specific bacteriologic etiology in necrosing arteritis, but should search for a specific immunologic phase or a specific sensitiveness of the blood vessels, and try to reproduce this, if possible, experimentally, instead of trying to reproduce the disease by the injection of organ extracts in such cases." While a specific allergic state has not been demonstrated in the studies here presented, there is a strong probability that such a state exists. In a review of the

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broader subject of primary inflammation of the arteries, Karsner [6] stated: "Degenerative and mild or severe inflammatory lesions of the arteries, not directly caused by an infective agent, are well known, but few if any correlative studies have been published." It is believed that a definite correlation exists in the studies here presented.

A brief summary of the events leading up to these arterial lesions will furnish the historical background and introduce the general methods employed. In an attempt to secure a test animal comparable to the patient with nephritis for testing the efficiency for new plasma protein production of various dietary and supplementary factors, 3 normal adult dogs, maintained on a standard low protein diet, were depleted to a basal plasma protein level of 4 per cent by repeated bleeding and return of the washed red blood cells suspended in a saline solution (plasmapheresis), and the rate of plasma protein production under these standard conditions was determined. Uranium nitrate was then injected subcutaneously and the effect of the ensuing elevation of nonprotein nitrogen upon plasma protein production was followed. Despite elevations in nonprotein nitrogen to more than ten times normal, no interference with plasma protein production was observed.[7] One unexpected finding, however, emerged from these studies: the dog with depleted plasma proteins is more susceptible to uranium nitrate injury than is the normal dog. Whereas the majority of normal adult dogs will survive the administration of 4.0 mg. of uranium nitrate per Kg.,[8] the 2 hypoproteinemic dogs receiving 3.0 and 2.5 mg. per Kg. both died in uremia in about 2 weeks. Only when the dose was reduced to 2.0 mg., or one half the usual amount, did a dog with depleted plasma proteins survive. This observation raised the question whether increase of the plasma protein concentration would protect against heavy metal poisoning. When the procedure was reversed, *i.e.*, when the dog's plasma protein level was increased from a normal of about 6.5 per cent to 9 or 10 per cent by repeated daily injections of plasma obtained from normal healthy donor dogs, and the dose of uranium nitrate was increased from 2.0 to 5.0 mg. per Kg., the arterial lesions to be described were found in all 5 dogs on which this procedure was tried.

Methods

All of the experimental animals were normal adult dogs. The dogs used as donors were large dogs maintained on the regular kennel diet of fresh meat and table scraps. A sufficient number of these were used so that anemia did not develop. The dogs in the experimental group were carefully selected. They were slender, well nourished (but not fat) dogs of hound or terrier extraction with readily accessible neck veins and femoral arteries, and were as small as practicable so that reductions or increases of the plasma protein level could be accomplished without using too great quantities of blood. The 8 dogs in this experimental group consisted of 5 females and 3 males whose estimated age varied from 1 to 5 years. All of the dogs in this experimental group were maintained on a standard basal diet which consisted of: calves' liver (raw wet weight) 32 parts, cane sugar 25 parts, corn starch 25 parts, butter 12 parts, cod liver oil 6 parts. Enough tomato juice was added to make a paste, of which each gram contained 3 calories. The diet was fed in amounts to furnish 75 calories per Kg. per day. One gm. of the McCollum-Simmonds salt mixture [9] and 5 gm. of kaolin were thoroughly mixed with each day's diet.

The details of methods for depleting the plasma proteins to standard hypoproteinemic level have recently been published.[7,10] The plasma injections, used in making the dogs hyperproteinemic, were carried out as follows: About 250 cc. of blood from a donor dog was bled into a flask containing 3 cc. of saturated sodium citrate and centrifugated for 30 minutes at 3500 r. p. m. in 100 cc. centrifuge tubes. The plasma was then drawn off with suction, warmed to 40 to 45° C. and injected into the jugular vein. Approximately 15 minutes was required for the plasma to run into the vein. Further details of this procedure can be found in previous publications.[11, 12]

Duplicate micro-Kjeldahl analyses of total nitrogen, nonprotein nitrogen and albumin plus nonprotein nitrogen (the filtrate from 22 per cent sodium sulfate precipitation by Howe's method) served as the basis for calculation of plasma protein removed or injected and blood level studies.[13]

The uranium nitrate ($UO_2(NO_3)_2.6H_2O$) was obtained from Merck, lot S.7497, and was injected subcutaneously in the groin

in 0.5 per cent aqueous solution. Sufficient water was used to insure quantitative transfer.

The experimental animals were kept under close observation, were sacrificed with ether if they were obviously moribund and were autopsied promptly after death. Routine sections, taken from all organs and from many of the tissues, were fixed in 4 per cent solution of formaldehyde and Zenker's solution. The Zenker's-fixed tissues were embedded in paraffin, sectioned at 7 μ, and stained routinely with hematoxylin and eosin. Special stains used in selected cases included: Verhoeff's stain for elastic tissue, sometimes combined with van Gieson's picro-acid fuchsin solution; Mallory's aniline blue stain for collagen; von Kossa's silver nitrate method for calcium; scarlet red stain for fats; and phloxine-methylene blue and Gram's stains for bacteria.

Experimental Observations

Table I gives in summary the experimental data on all of the dogs which developed arterial lesions. The anatomical distribution of these lesions is indicated in Table II. In Table I the dogs are divided into three groups and the same grouping is indicated in Table II.

Group A comprises the 5 dogs rendered hyperproteinemic by repeated daily injections (17 to 24 in the course of 3 to 4 weeks) of plasma obtained from healthy donor dogs. The dose of uranium nitrate in each was 5.0 mg. per Kg. In all but one of these dogs sodium citrate was used to prevent coagulation in obtaining the donor's plasma. Thus each daily injection, which averaged 100 cc., contained 2 to 3 cc. of saturated sodium citrate. In 1 dog (No. 40–50) heparin (50 mg. per injection) was used. The lesions in this dog were possibly a little less marked than in the dogs receiving citrate, but they were qualitatively the same.

Group C consists of 2 of 4 dogs made hypoproteinemic by almost daily withdrawals of plasma (plasmapheresis) over a period of time similar to that in Group A. In about one half of the instances, the experimental dog was bled directly and, after removing the plasma from the centrifugalized, citrated blood with the aid of suction, its own cells, resuspended in a saline solution, were reinjected. At other times, immediately after bleeding the experimental animal, the previously prepared corpuscles of a

donor, suspended in a saline solution and warmed to body temperature, were injected. Regardless of which procedure was used, after drawing off the plasma, the red blood cells were always washed once with modified Locke's solution [14] before they were injected. Analysis of these first "washings" have shown that they contained only about 7 per cent as much protein as the original plasma; hence this procedure removed over 99 per cent of the plasma protein but did not remove all of this protein.[14] This point is emphasized, for it is possible that the recipient is sensi-

TABLE I

*Summary of Experimental Data on Dogs with Arterial Lesions**

Group†	Dog number	Number of injections or exchanges‡	Total amount of plasma injected or removed	Plasma protein concentration		Body weight§	Uranium nitrate injected subcutaneously	Height of non-protein nitrogen‖	Survival interval¶
				Before alteration	After alteration				
			cc.	*gm./100 cc.*	*gm./100 cc.*	*Kg.*	*mg./Kg.*	*mg./100 cc.*	*days*
	39–34	22	+2655	6.5	9.4	4.4	5.0	297	8
	39–40	17	+2010	7.0	8.8	6.5	5.0	582	11
A	39–45	22	+2210	7.1	9.2	7.3	5.0	617	15
	40–50	24	+1880	6.6	10.0	4.8	5.0	573	17
	39–28	24	+2225	7.2	9.9	5.1	5.0	92	335
B	39–43	2	+ 125	6.6	6.1	4.7	5.0	438	15
C	39–49	17	−1492	8.2	4.0	4.8	5.0	391	15
	38–24	20	−2040	7.6	4.1	4.5	3.0	386	15

* All dogs were maintained on basal diet throughout entire period.
† Group A, hyperproteinemic; Group B, see text for discussion of this dog: Group C, hypoproteinemic.
‡ The total period in which these injections were made was 3 to 4 weeks.
§ Body weight at end of period of plasma injections or withdrawals, *i.e.*, weight on day of uranium nitrate injection.
‖ In all instances except dog No. 39–28 which survived the acute stage, the nonprotein nitrogen as recorded is on a sample taken immediately prior to death or during the previous day.
¶ Interval between injection and autopsy.

tized to some constituent of plasma obtained from one or more of the donor dogs.

The other 2 dogs in this hypoproteinemic group (Nos. 38–25 and 39–27 [7]), although the experimental procedure was identical, did not develop arterial lesions and therefore are not included in this paper. The only known difference for these dogs was that the dosage of uranium nitrate was less: in No. 38–25 it was 2.5 mg. per Kg. and in No. 39–27, 2.0 mg. per Kg. Thus all dogs in all groups (see following for Group B) which in addition to some alteration in their plasma proteins received 3.0 mg. or more of

uranium nitrate per Kg., developed arterial lesions; but a much larger number of animals with varying doses of uranium nitrate would be necessary before one could feel confident that this statement would continue to hold true.

Group B consists of only 1 dog which was given 2 injections totaling 125 cc. of citrated plasma obtained from donors on 2 successive days 3 weeks before the injection of 5.0 mg. of uranium

TABLE II

*Anatomical Distribution of Arterial Lesions**

Dog No.	Aorta		Sinuses of Valsalva	Coronary arteries	Left auricle	Pulmonary artery	Arteries in lungs	Other arteries
	Ascending	Abdominal						
39-34	+++	++	++	+		+++	+‡	Mesenteric +
39-40	+++†	++	++		+++†	+++		Mesenteric +, carotid +
39-45	++‖	+++†				++	+	
40-50	+			+		++	+	Mitral valve +
39-28	+					+++†	+	All lesions "healed"
39-43	++			+	+++	++	+	
39-49	+		+	+	++			Mitral valve ++
39-24	+++	+++‖	++	++		+++	+	Innominate++, ‖; lingual ++,‖; femoral ++,‡‖; subclavian ++,‖; internal mammary ++; mesenteric +.

* The lesions have been graded as follows: +++ = gross lesion over 1 cm. in maximum diameter; ++ = gross lesion less than 1 cm. in maximum diameter; + = lesion discovered in microscopic sections.
† Gross and microscopic illustration.
‡ Microscopic illustration.
‖ Thrombus associated with lesion.

nitrate. Because of "nervousness" and "difficult" neck veins this dog was dropped from the hyperproteinemic group and shifted over to a control group. Since, of the 6 control dogs, this was the only one that developed lesions, it is believed that these two injections of donor's plasma are significant.

This separation of the experimental animals into groups is useful only in presenting the experimental data and in discussing the possibilities of pathogenesis, for no such separation into groups is possible from an anatomical study of the lesions.

Clinical evidence pointing to the arterial lesions was encountered in only one dog, and in this case the symptoms and signs were referable to thrombi which formed over the arterial lesions. During the 48 hours preceding death, dog No. 38–24 developed progressively gangrene of the anterior third of the tongue and paralysis of both hind legs and the right foreleg. The affected extremities were cooler than normal and no arterial pulsation was palpable in them. None of the other 7 dogs showed any clinical manifestations referable to the arterial lesions. The chief cause of death in all instances was presumably "uremia." The figures for the terminal nonprotein nitrogen and the extensive necrosis of the proximal convoluted tubules seen in histological sections of the kidneys substantiate this clinical impression.

Gross and Microscopical Description

As indicated in Table II, the lesions were found principally in the aorta, sinuses of Valsalva, pulmonary artery and in the endocardium of the left auricle and were strikingly absent from the parenchyma of organs other than the lungs and heart. Involvement of the coronary arteries was confined to the larger branches. The innominate, carotid, subclavian, internal mammary, femoral and mesenteric arteries were examined in all but one of the dogs. Gross lesions were found in only one dog (No. 38–24) but as indicated in Table II, histological lesions were found in the mesenteric artery in two other dogs and in the carotid artery in one of these. Thus both elastic and muscular arteries were subject to the injury, but the lesions were more prevalent and more prominent in the elastic arteries. (The structure of the left auricle warrants its inclusion in the category of elastic arteries.) No lesions were found in veins or lymphatics.

The lesions in the ascending aorta, sinuses of Valsalva and pulmonary artery were similar in gross and histological appearance. When the vessels were opened the lesions appeared on the intimal surface as slightly raised, wrinkled, inelastic, grayish brown patches which contrasted sharply with the smooth intima about them. They varied in size from 1 to 2 mm. to 2 to 3 cm. in maximum diameter and were sometimes confluent (Fig. B). Frequently they were round or oval in outline and sometimes they were sharply defined and apparently limited to the intima (Fig.

1). Occasionally, however, the outlines were irregular and in some instances extension into the media could be seen with the unaided eye. Engorged tiny vessels, hemorrhage and a scant amount of fibrin could sometimes be detected in the adventitia. Thrombosis was the exception rather than the rule. No gross evidences of calcification were encountered in these regions. In the aorta the lesions were limited to the ascending portion, the first half of the arch and the abdominal aorta. The thoracic portion of the descending aorta was not involved. The lesions were usually most marked in the first portion of the ascending aorta. Occasionally they completely surrounded the orifice of one of the coronary arteries, but in no instance was there any narrowing of the orifice. The sinuses of Valsalva and of the pulmonary valve, when involved, appeared bulged outward and irregularly wrinkled or showed only one or two small grayish brown, raised areas. In the pulmonary aorta, as in the ascending aorta, the lesions were more pronounced in the first portion and did not extend beyond the bifurcation. The lesions in the lungs (Fig. 12) were found only microscopically.

Under the microscope it was difficult to determine just how the process began. There appeared to be an injury to the subendothelial connective tissue with pyknosis of the nuclei and swelling of the cells and fibers in this tissue (Fig. 2). No doubt there was also some injury to the endothelial lining with increase of its permeability, for edema, fibrin, and hemorrhage soon made their appearance. Subendothelial deposits of fibrinoid material were sometimes seen (Fig. 3). The fully developed process, which was the one usually encountered, presented massive necrosis with destruction of the intima, often extending deeply into the media, and there was usually some inflammatory reaction in the adventitia. The predominant recognizable leukocyte in this reaction is the polymorphonuclear neutrophil, but the majority of the cells are most accurately described as "cells with distorted nuclei." Fully developed eosinophils were not seen and only occasional eosinophilic myelocytes were encountered. In the more advanced lesions, large mononuclear cells were numerous and there was some enlargement, basophilic staining and proliferation of the fixed tissue cells (fibroblasts and endothelial cells), indicating attempts at repair. The destructive changes, including massive

karyorrhexis and karyolysis with the formation of pools of nucleic acid, so far overbalanced the proliferative changes that the finding of healed lesions in the pulmonary artery of dog No. 39–28 (Fig. 7) was a distinct surprise. The gross and especially the histologic changes suggested an aneurysm as a more likely possibility but no definite aneurysms were encountered. Other surprising features were that the endothelial lining remained intact as long as it did and that thrombi were not more frequent.

Elastic tissue stains showed that in the earliest lesions the elastic framework was intact, but instead of the usual wavy contour the fibers were straight. Very soon, however, the internal elastic membrane became frayed and fragmented and as the lesion progressed, bits of the fragmented elastic fibers broke off into the necrotic debris and apparently underwent lysis. In the most extensively involved area, the elastic framework of practically the entire thickness of the media of the aorta had been destroyed. Figure 10 illustrates the margin of this area. Fat stains were negative. Von Kossa stains have revealed the presence of tiny black calcium granules along the course of some of the disintegrating elastic fibrils, but these were not so numerous as the hematoxylin and eosin preparations suggested. Numerous Gram and methylene blue stains of the most promising lesions have failed to reveal any organisms. The connective tissue stains, both van Gieson's and Mallory's, showed about equal involvement of smooth muscle and collagenous tissue.

In the endocardium of the left auricle the lesions appeared as tiny, punctate or streaky, slightly raised, yellowish opacities and were usually grouped in an area 1 to 2 cm. in diameter above the posterior leaflet of the mitral valve (Fig. D). In one case similar lesions were present in the left auricular appendage. In all three instances in which lesions were found in this chamber, the endocardium over them appeared to be intact and there was no adherent thrombus. Subendocardial hemorrhages were not infrequent in the left auricle but were usually more numerous in the left ventricle and have been found in the mitral and aortic valves (Fig. B). Hemorrhages were also encountered in the epicardium, especially along the right atrio-ventricular groove and about the base of the aorta.

Histologically the lesions in the endocardium of the left auricle

were similar to those described (Figs. 4–6). Possibly because the tissues in this region were less compact than those in the aorta, the deposition of fibrin and fibrinoid material and the infiltration of leukocytes was a little more conspicuous. Similar, though less marked, exudate sometimes extended out into the interstitial tissue of the subjacent myocardium. While the cardiac fibers themselves were not usually involved, in two of the dogs a rather extensive necrotizing myocarditis involving the septum, papillary muscles and wall of the left ventricle, was encountered. Also in two instances the mitral valve showed acute necrotizing valvulitis. Collections of large mononuclear cells, sometimes mixed with polymorphonuclear leukocytes, occurred in the perivascular connective tissue of the myocardium, but none of these collections had the characteristics of an Aschoff body.

In the abdominal aorta (Fig. A) and in some of the other sites tabulated in dog No. 38–24 (innominate, subclavian, femoral, and internal mammary arteries), the gross appearance was somewhat different. Even before the vessels were opened, marked irregularity was obvious. The crumpled, inelastic, irregularly dilated and sometimes beaded appearance of the vessels contrasted sharply with the normal tubular or uniformly collapsed appearance. On opening the vessels, the crumpled, irregularly dilated character was even more manifest; and as the vessels were opened the scissors occasionally gritted as though cutting through very thin egg shells. The endothelium was intact save at the sites of thrombosis (innominate and both femoral arteries); and when viewed from the intimal surface, the vessel wall was less translucent than normal.

On histological examination of these vessels, the outstanding features were necrosis and deposition of calcium. At once was noted the loss of the normal wavy pattern of the internal elastic membrane. The normal folds of the intimal surface of these arteries, as seen in cross section, were obliterated. This change may involve only a portion or the entire circumference of the vessel. The nuclei in the media were fading or gone and the tissue in this region either stained more intensely with eosin or was becoming hyalinized and impregnated with calcium salts. Figure 11 shows an intense deposit of calcium in the necrotic media of one of the femoral arteries. The lumen at this site was completely

occluded by a thrombus. In and about some of these necrotic areas were appreciable infiltrations of polymorphonuclear leukocytes, apparently coming both from the lumen and from the vasa vasorum, but in most of the sections examined, necrosis and calcification overshadowed leukocytic infiltration.

In only one instance, dog No. 39–28, was there an opportunity to examine other than the acute lesions described above, and in this case there was a complication which may invalidate the findings. Despite the fact that this dog was subjected to the same experimental procedures as the other dogs in Group A; *i.e.*, the same basal diet, repeated injections of plasma obtained from the same group of donors, and the same dosage (5.0 mg. per Kg.) of the same standard solution of uranium nitrate, at no time was there any clinical evidence of illness. The usual proteinuria followed the injection of the heavy metal and by the eighth day the nonprotein nitrogen had risen to 92 mg. per 100 cc., yet the dog continued to eat all of its diet each day, body weight was maintained, and the general clinical condition was good throughout. The dog was kept on the basal diet for 9 weeks, by which time the plasma protein concentration had dropped to 6.6 per cent and the nonprotein nitrogen had fallen to 36 mg. per 100 cc. It was then returned to the regular kennel diet until it was sacrificed with ether 11 months after the uranium nitrate injury and almost exactly 1 year after the first injection of donor's plasma. At autopsy, the remarkable lesions depicted in Figure C were found in the pulmonary aorta. There were literally hundreds of tiny grayish white tags, everywhere covered by smooth white endothelium, which gave the intimal surface a shaggy appearance. It is noteworthy that no thrombi formed over this very irregular surface.

The complication in this dog consisted of the presence of four living adult female "heart worms" (*Dirofilaria immitis*) coiled up in the lumen of the pulmonary artery. Other cases of this infection seen in this laboratory and cases reported in the literature [15] have not shown the anatomical changes illustrated in Figure C. Hence it is reasonable to assume that there had been previously an acute arteritis similar to that which appeared in the other 4 dogs in this group and that it had "healed" with the residual fibrous intimal "scars" shown in Figures 7 and 9. These "scars" were confined to the intima and consisted of fibrous tissue with

only a scant infiltration of lymphocytes and large mononuclear cells persisting. The endothelial surface over these "scars" was everywhere intact. There were no capillaries and no old phagocytosed blood pigment in any of the "scars." The media and adventitia appeared entirely normal. Several of the arteries in the lungs showed similar fibrous intimal thickenings (Fig. 8), and there were a few areas of fibrosis about the vasa vasorum and in the interstices of the elastic framework of the aorta. The internal elastic membrane in the aorta in all the sections examined was normal, and there were no fibrous intimal thickenings in this vessel. The endocardium of the left auricle and all other blood vessels in the routine sections showed no change. The question whether the arterial lesions of dog No. 39–28 were due to the healing of a previous necrotizing arteritis or were the result of intimal reaction to the parasitic infection, or a combination of the two, must be left open. The idea that these acute lesions can heal is not incompatible with the findings in some cases of periarteritis nodosa.[16]

Discussion

The questions of most interest concern the pathogenesis of the lesions and their possible bearing on lesions encountered in human pathology. In regard to the latter point, about all that can be said at this time is that some of the lesions bear some similarity to those of periarteritis nodosa and rheumatic arteritis, but no claim of identity is made. The discussion of the former point is divided into three parts:

1. *Exclusion of certain possibilities of error.* Are the lesions related to the experimental procedure or are they merely coincidental? To rule out the possibility of an epidemic or epizootic of some sort (such as that recently described by Loewe and Lenke [17] in rabbits, with cardiac lesions resembling those of human rheumatic fever), 20 dogs, derived from the same sources and kept in the same quarters at the same time as the experimental animals, were examined. Not one of these had any lesions in its arteries. Furthermore, despite the fact that these experiments have been in progress for over a year, both the gross and histologic appearance of the lesions has in all instances corresponded to the time interval between the injection of uranium nitrate

and death: 8 to 17 days for 7 of the dogs and 11 months for dog No. 39–28.

Are the lesions due to something accidentally introduced during the experimental procedure, such as a bacterium, virus, or protozoan? This question, which is especially pertinent since sterile technic was not employed in making the plasma injections or withdrawals, cannot be answered finally; but all available evidence is against this possibility. (a) In dogs inoculated with known bacteria, viruses, or protozoa, such arterial lesions do not develop. (b) Blood cultures of the experimental dogs have been negative. (c) Sections of the lesions stained with bacterial stains have failed to reveal any organisms. (d) Intravenous plasma injected alone, plasmapheresis alone, or subcutaneous injections of uranium nitrate alone do not produce the lesions, and the opportunity of introducing organisms or virus along with these injections is just as great as it is in the experiments reported. These facts seem to exclude every possibility except the accidental introduction of some unknown organism or virus having a peculiar affinity for arteries.

2. *Negative evidence bearing on pathogenesis.* Since all the dogs with arterial lesions were maintained on the standard basal diet throughout the experimental procedure, it is conceivable, although unlikely, that some constituent of the diet enters into the reaction which produces the arterial lesions. (Viosterol lesions in rabbits [18] pointed the finger of suspicion at the cod liver oil which constituted 6.5 per cent of the diet by weight.) To check on this point, 6 dogs were placed on the standard basal diet for 4 weeks and then given 5.0 mg. of uranium nitrate per Kg. Only 1 of the 6 dogs developed arterial lesions and, as previously explained, this dog (No. 39–43) had received 2 intravenous injections of donor's plasma 3½ weeks previously; hence was not a strict control. This dog has been included in the experimental group (Tables I and II), leaving the 5 unequivocal control animals entirely negative. This evidence, of course, does not mean that the diet or some constituent thereof is not a necessary part of the reaction which produces the arterial lesions. This point is being checked at the present time.

Since the anticoagulant used in obtaining donor's plasma is injected into the vein of the experimental animal along with the

plasma, it is conceivable that it might play a rôle in the production of the lesions. In 4 of the dogs in the hyperproteinemic group (group A), sodium citrate was used for this purpose. In the other member of this group, dog No. 40–50, heparin was used as the anticoagulant. Identical arterial lesions appeared in this dog. This, plus the fact that lesions developed in 2 hypoproteinemic dogs (group C) in which the amount of anticoagulant injected along with the *washed* corpuscles was negligible, seems to exclude this factor from any pathogenic rôle.

3. *Conjectures on pathogenesis.* No plausible theory of pathogenesis has as yet been developed and no direct evidence of any kind has been obtained. The feature which is most impressive as having some pathogenic significance is the histologic appearance of the lesions. Their similarity to those encountered in anaphylactic and allergic states is striking, but with one notable difference. In these altered states, the acute necrotizing changes are found predominantly in the smaller blood vessels and capillaries and not in the larger arteries and endocardium, as in the experiments reported in this paper. Furthermore, abnormalities correlated with repeated daily injections over a period of 3 to 4 weeks suggest hypersensitivity or altered sensitivity as a mechanism of their production. The possibility that the lesions are due to incompatibilities (such as those described by Melnick, Burack and Cowgill [19] in dogs receiving repeated infusions of red blood cells, or those described by Rous and Robertson [20] in rabbits receiving repeated small transfusions) developing from some constituent of the blood of one or more of the donor dogs has to be considered. Records were kept of all bleedings and injections and these were checked with this possibility in mind, but no incriminating evidence was obtained. Although 2 or more of the 10 donor dogs were common to 6 of the experimental animals with arterial lesions, there is no single donor common to all the experiments. No direct observations on cross-matching at different stages in the experimental procedure have been made. Certainly the negative evidence just cited does not exclude the possibility of incompatibilities. But even if positive evidence on this point were obtained, the rôle of uranium nitrate in the production of the lesions would still remain unexplained.

Uranium nitrate could act in one or more of several ways. It

could act as a simple chemical compound to form a "conjugated protein" [21] with some constituent of the blood of one or more of the donor dogs; and this "conjugated protein" could act as sensitizing antigen. Or, uranium nitrate could precipitate the "reaction" previously prepared by some constituent of the blood of one or more of the donor dogs. Or, the essential action of uranium nitrate could be the production of renal injury, and the essential mechanism in the production of the arterial lesions could be some constituent in the blood of one or more of the donor dogs (or product thereof) acting in the disturbed metabolism consequent to the renal injury. It is of interest in this connection that a considerable percentage of human cases of generalized necrotizing arteritis are associated with diffuse glomerulonephritis.[5]

Summary of Discussion

The arterial lesions presented in this paper in all probability are not due to accidental or coincidental infection, but seem definitely to be related to the experimental procedure. Diet and anticoagulant can probably be excluded from any pathogenic rôle. The factors common to all of the experiments in which arterial lesions developed are: (1) some alteration in the plasma proteins produced by the intravenous injection of some constituent of apparently "homologous" blood, and (2) uranium nitrate injury (3.0 mg. or more per Kg. administered subcutaneously 3 to 4 weeks after starting the alteration). As a tentative hypothesis it is suggested that the lesions may be due to a hypersensitivity to some constituent of the blood of one or more of the donor dogs precipitated by uranium nitrate or made manifest in the disturbed metabolism consequent to the uranium nitrate injury.

Summary

1. During experiments designed to determine whether heavy metal poisoning is influenced by altering the plasma protein level it was observed that 8 of 10 dogs, subjected to alterations in their plasma proteins by plasmapheresis or plasma injections and then given uranium nitrate subcutaneously, showed necrotizing arteritis when they died 8 to 17 days later. One of the 8 dogs survived the early phase of the experiment and showed healed

lesions in the pulmonary artery when it was sacrificed 11 months later.

2. Control dogs, maintained on the same diet and receiving the same dosage of uranium nitrate (5.0 mg. per Kg.) but not subjected to plasmapheresis or plasma injections, failed to show any arterial lesions.

3. The lesions affected principally the elastic arteries, the commonest sites being the aorta, sinuses of Valsalva, pulmonary arteries and the endocardium of the left auricle. These lesions are strikingly absent from the parenchyma of organs other than the heart and lungs.

4. Essentially, the lesion is an acute necrotizing arteritis usually most marked in the intima but not infrequently involving all three coats. Polymorphonuclear neutrophils predominate in the cellular response. Calcium deposition in the necrotic areas is frequent. Sometimes thromboses occur. Some of the lesions have a semblance to those of periarteritis nodosa and rheumatic arteritis.

5. Bacteriological studies of the blood and bacterial stains of the lesions have failed to reveal any organisms.

6. The possibility of a spontaneous disease, epizootic in nature, seems to be excluded by the control data.

7. The nature of the process is briefly discussed.

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DESCRIPTION OF PLATES

PLATE 69

Necrotizing arterial lesions in dogs.

FIG. A. Dog No. 39–45. Necrotizing aortitis of abdominal aorta.

FIG. B. Dog No. 39–40. Acute necrotizing aortitis of ascending aorta. There is hemorrhage in the septal endocardium extending into the base of the anterior aortic cusp.

FIG. C. Dog No. 39–28. Healed necrotizing arteritis of the pulmonary aorta.

FIG. D. Dog No. 39–40. Acute necrotizing left auriculitis.

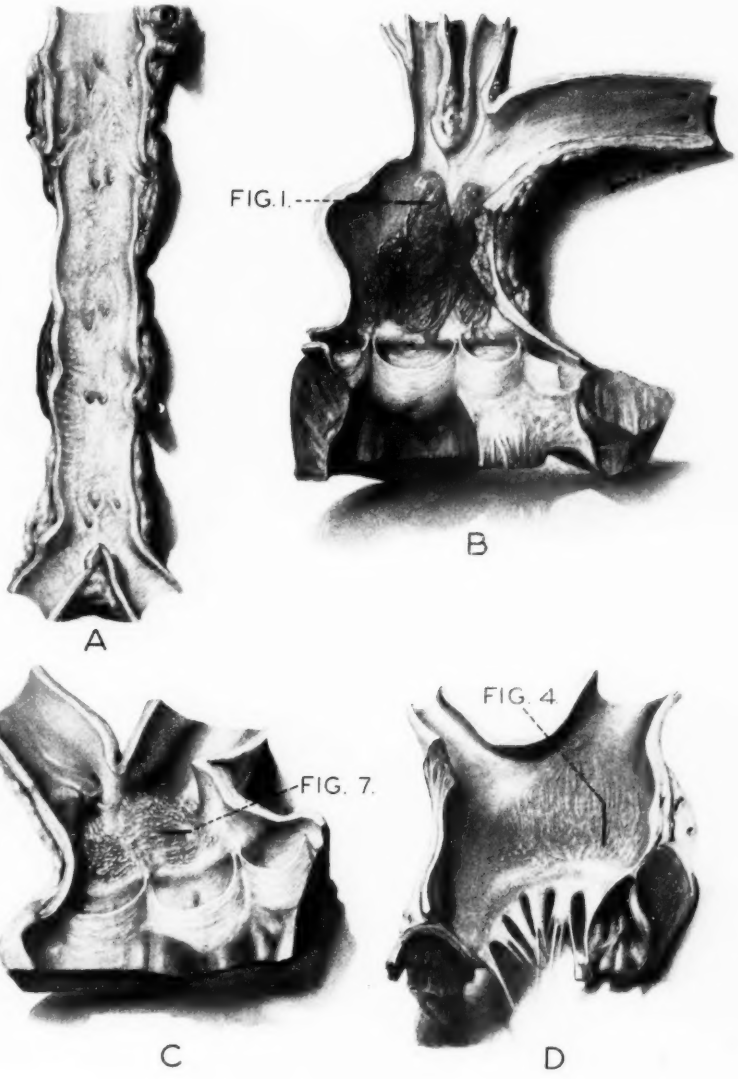


FIG. 1.
A
B
FIG. 7.
FIG. 4
C
D

PLATE 70

Necrotizing arterial lesions in dogs. All sections were stained with hematoxylin and eosin.

FIG. 1. Dog No. 39–40. (See Figure B for site of section.) Acute necrotizing aortitis largely confined to the intima and the inner layers of the media. Note the intact endothelium and sharp border of lesion. × 45.

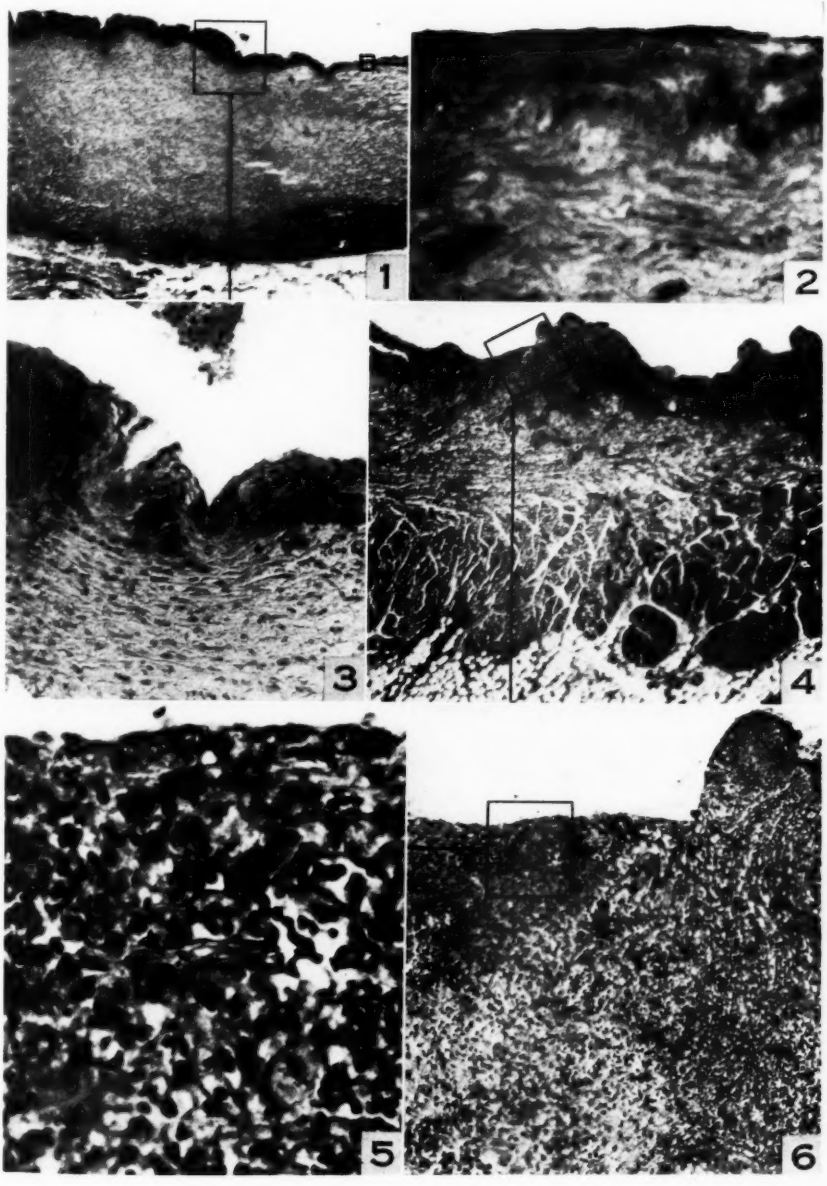
FIG. 2. Dog No. 39–40. (From area indicated in Figure 1.) Necrosis and swelling of subendothelial collagenous tissue. There is also some edema. × 610.

FIG. 3. Dog No. 39–40. (From area indicated in Figure 1.) Deposition of fibrinoid material beneath intact endothelium. Massive necrosis and infiltration of leukocytes, both polymorphonuclear and mononuclear, and cells with distorted nuclei. × 150.

FIG. 4. Dog No. 39–40. (See Figure D for site of section.) Acute necrotizing auriculitis. Massive leukocytic infiltration and fibrin deposition beneath the intact endothelium; also some polymorphonuclear infiltration of the interstitial tissue of the myocardium. × 45.

FIG. 5. Dog No. 39–40. (From area indicated in Figure 6.) High power view of the exudate. Cells with distorted nuclei predominate, but polymorphonuclear leukocytes and large mononuclear cells can be recognized. Note the mitotic figure in one of the latter cells near the right lower corner. × 610.

FIG. 6. Dog No. 39–40. (From area indicated in Figure 4.) Medium power showing intact endothelium, fibrin deposition, leukocytic infiltration and necrosis. × 110.



Holman

Experimental Necrotizing Arteritis

Plate 71

Necrotizing arterial lesions in dogs. All sections, except that used for Figure 10, were stained with hematoxylin and eosin.

Fig. 7. Dog No. 39–28. (See Figure C for site of section.) Fibrous intimal plaques covered with endothelium. These are presumably healed lesions (see text). × 40.

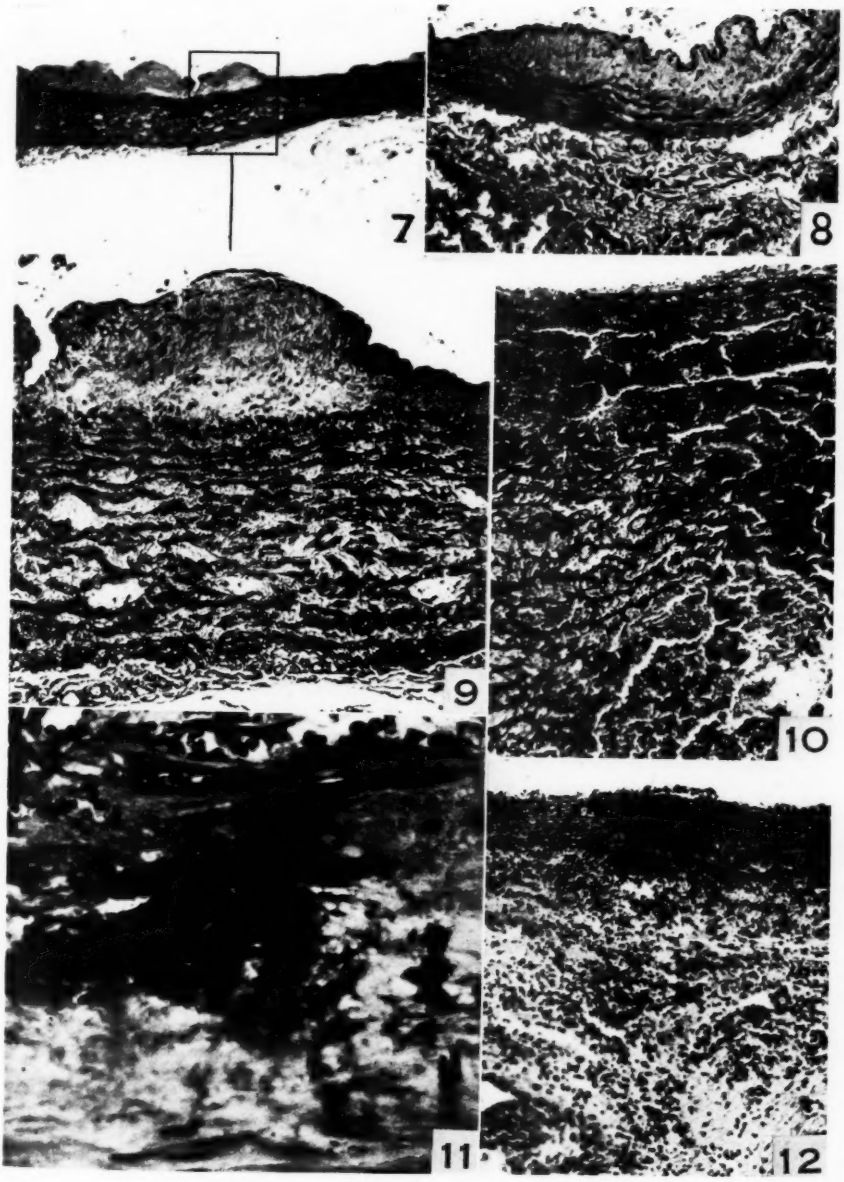
Fig. 8. Dog No. 39–28. Similar fibrous intimal plaque in artery in lung. × 110.

Fig. 9. Dog No. 39–28. (From area indicated in Figure 7.) × 150.

Fig. 10. Dog No. 39–40. Acute necrotizing aortitis showing disruption of elastic framework and leukocytic exudate in deeper layers of media. Verhoeff's elastic tissue stain. × 100.

Fig. 11. Dog No. 38–24. Necrosis, hyalinization and calcium deposition in the media of the femoral artery. The lumen of this vessel was occluded by a thrombus. × 610.

Fig. 12. Dog No. 39–34. Acute necrotizing arteritis and periarteritis in an artery in the lung. Note loss of elasticity (as indicated by straight contour of vessel), massive leukocytic infiltration and necrosis. × 110.



Holman

Experimental Necrotizing Arteritis

THE CYTOLOGIC RESPONSE OF RATS AND MICE TO A STRAIN OF GREENING STREPTOCOCCI*

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Numerous experiments, designed to test the etiologic relationship of strains of greening streptococci to rheumatic heart disease, have centered attention on focal collections of mononucleated cells in the myocardium found particularly in rabbits. Some investigators [1-4] maintained that these cellular aggregates resemble Aschoff bodies so closely as to be identical with them, whereas others [5-8] thought that these inflammatory lesions differ from those of rheumatic fever and that any resemblance to Aschoff bodies was without diagnostic significance.

The present study of myocardial lesions with associated changes in other organs in rats and mice, following infections with greening streptococci, is reported because it seems to throw additional light upon the significance of such findings in other animals.

MATERIALS AND METHOD

A strain of greening streptococci, recently isolated from a case of subacute bacterial endocarditis, was grown on neopeptone beef broth of P_H 7.6 for 48 hours and used without previous animal passage. Intracardiac injections of this organism were given three to five times a week to 25 rats weighing from 125 to 150 gm. The material injected consisted of the centrifugalized bacterial sediment from 0.6 cc. of a 24-hour culture resuspended in 0.3 cc. of the supernatant broth. The number of such injections varied from 4 to 19 per rat and the life of the animals varied from 4 to 58 days. The intracardiac injections were done under light ether anesthesia with a tuberculin syringe and a 26 gauge needle. The site of puncture was prepared by wetting the hair and skin with alcohol. Some of the rats given multiple intracardiac injections died of cardiac tamponade during the course of the injections; others died spontaneously of the induced disease; whereas the remainder were killed with ether.

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The following controls were employed: 15 rats were given a single intracardiac injection of the same bacterial suspension and were killed at intervals varying from 6 hours to 11 days; 5 rats were given 2 to 4 intracardiac injections of heat-killed organisms; and 5 others were similarly injected with sterile broth. All control animals were killed with ether 4 to 8 days after the first injection.

A group of 14 mice weighing approximately 20 gm. received 16 injections, each consisting of 0.1 cc. of a similarly concentrated culture, by the tail vein, during a period of 3 months. Only 3 of the mice died of intercurrent infection prior to the conclusion of the experiment.

The heart's blood of all rats and mice was cultured in broth after autopsy and paraffin sections of heart, brain, kidneys, and lungs were prepared following fixation in formaldehyde solution. The sections were stained with hematoxylin and eosin. Bacteria were demonstrated by the Gram stain, collagen by Masson's stain, and reticulum by the method of Wilder.

Results

The early reaction (24 to 48 hours) in rats consisted of miliary abscesses in the myocardium, kidneys and brain. The myocardial abscesses in a few animals were associated with diffuse leukocytic infiltration. A diffuse leukocytic infiltration was also present in the epicardium of many rats, particularly at the auriculoventricular sulcus.

Following this initial response there was a rapid replacement of leukocytes by monocytes in the involved organs. This was particularly pronounced in rats which received only one intracardiac injection. Animals given multiple injections exhibited relatively few leukocytes in the myocardium 6 days after the initial injection, and in several instances as late as 17 days after the first intracardiac injection. It was interesting to note that a number of rats given multiple injections failed to exhibit a leukocytic response in the heart or elsewhere, although no more than 24 to 48 hours had elapsed since the last intracardiac injection.

More than one third of the rats given four or more intracardiac injections exhibited myocardial lesions which were not found in control animals given only one injection. The lesions, usually in

interstitial and occasionally in perivascular regions in the ventricular myocardium, consisted of focal collections of large, epithelioid, nonphagocytic cells with abundant, somewhat basophilic cytoplasm, poorly defined cell boundaries and large oval nuclei. A heavy nuclear membrane and a prominent, frequently stellate nucleolus contributed to the close resemblance to the cell described as characteristic of the Aschoff body (Fig. 1). However, remnants of degenerated or necrotic collagen in these foci and the "chicken-wire" network of reticulum stressed by Louis Gross and Ehrlich [9] as important criteria of the Aschoff body, were not demonstrable in sections stained specifically for these substances.

Valvulitis and mural endocarditis, often with massive vegetations, were encountered in 10 (40 per cent) rats subjected to multiple intracardiac injections (Fig. 2). The vegetations consisted of fibrin, leukocytes, debris, and numerous bacterial masses. Varying degrees of organization were present near the endocardial attachment in some vegetations. In 2 rats an acute purulent endaortitis was seen a short distance above the valve.

Myocardial scars were the most constant finding in nearly all rats 4 or more days following single or multiple intracardiac injections with living or heat-killed streptococci, or with sterile broth. The character of the scar varied, of course, with its age. Animals that survived 2 weeks or longer exhibited well-contracted, collagenous scars containing some hemosiderin, practically no inflammatory cells, and few adult connective tissue nuclei.

The evanescence of the leukocytic response to this strain of greening streptococci was also demonstrable in the brain. Whereas masses of leukocytes surrounding bacterial clumps were characteristic of brain lesions 24 hours old, 4 days later the leukocytes were nearly all replaced by monocytes and the bacteria had completely disappeared (Fig. 3). Rats that lived 9 or more days after the initial injection and were subjected to numerous subsequent injections exhibited no lesions which could be identified by the type of cell present as of more recent origin; nor were the observed lesions per section more numerous in animals which received 15 intracardiac injections than in those which had been injected only once or twice. At this time the brain lesions consisted of small clumps of large monocytic phagocytes which

contained hemosiderin and were situated in areas where there was an apparent loss of brain substance. Some extracellular hemosiderin was also present.

The kidneys of rats given single infective doses showed lesions qualitatively identical with those of animals subjected to multiple injections. The latter exhibited interstitial inflammatory foci in addition to a classical focal embolic glomerulonephritis. The leukocytes present 24 hours after injection were quickly replaced by monocytes; so that 5 or more days after the initial injection, most animals exhibited only healing or healed glomerular lesions (Fig. 4) in spite of numerous superimposed, relatively recent intracardiac injections.

Although bronchitis, peribronchitis, and pneumonia were not uncommon findings in both infected and control rats, 10 of 25 which received multiple intracardiac injections of living organisms showed lesions not seen in other animals. These lesions were composed of mononucleated cells and Langhans' giant cells grouped to form miliary nodules which contained no bacteria.

Rats injected with sterile broth or with heat-killed cocci showed no lesions other than occasional myocardial scars.

Three fourths of the mice given repeated intravenous injections with suspensions of greening streptococci exhibited focal monocytic infiltrations of the myocardium but no endocardial vegetations or foci resembling Aschoff bodies. Occasional myocardial scars and, more frequently, focal epicardial infiltrations by monocytes and lymphocytes were additional findings. Less than one half of these mice showed brain lesions. These consisted of small areas of degeneration or necrosis infiltrated by monocytes and occasional leukocytes. Occasional vessels exhibited "collars" of similar cells. Although focal, embolic, glomerular lesions were not found in the injected mice, nearly all exhibited small, interstitial, monocytic foci.

Discussion

The salient result of multiple intracardiac injections of greening streptococci was the monocytic reaction in the heart, brain, kidneys and lung, which was preceded by a transient leukocytic response. Focal myocardial lesions resembling Aschoff bodies were observed in rats but not in mice. These lesions differed from

true Aschoff bodies in several important respects and appeared to be merely a slight modification of the monocytic response provoked by the greening streptococci in other organs. The peculiar miliary nodules of monocytes and Langhans' giant cells seen in the lung represented still another modification of the same monocytic response.

A mobilization of large monocytes in association with Langhans' giant cells was described by Nye and Parker [10] following repeated intravenous injections of nonhemolytic streptococci in rabbits. The experimental results obtained by Louis Gross, Loewe and Eliasoph [8] led these workers to conclude that "the rabbit and possibly other animals, are peculiarly prone to the formation of multinucleated cells in collections somewhat resembling Aschoff bodies following the injection of a variety of toxic substances."

This statement may be paraphrased to the effect that the monocytic response seen in the rat, rabbit, and probably other animals following the injection of greening streptococci is characteristic of the reaction of an immune or partially immune host to a pathogen of relatively low virulence; and that the formation of giant cells as well as of structures resembling Aschoff bodies is merely a minor modification of this general reaction.

Indications of such immunity are seen in the failure of most rats to respond with new abscesses in the various organs following repeated intracardiac injections of living streptococci.

No particular significance or importance is attached to the presence of endocardial vegetations in 28 per cent of the rats. Various workers have produced endocardial vegetations in dogs and rabbits by a combination of cardiac trauma with intravenous injections of greening streptococci and in this respect the production of vegetations in these rats was similar.

The cytologic response to greening streptococci in mice was qualitatively similar to that seen in rats, except that lesions simulating Aschoff bodies and miliary nodules in the lungs were not observed. Endocardial vegetations were not found in mice, probably because the heart was not traumatized. The cellular reaction in the rat was more severe than in the mouse, probably because the trauma of the intracardiac injections often established foci which favored a more lasting bacteremia than existed with a simple intravenous injection.

Summary

The cytologic response in the rat to intracardiac injections of living cultures of greening streptococci was predominantly monocytic, preceded, however, by a transient leukocytic reaction. In the myocardium of many animals, structures resembling Aschoff bodies were seen. This resemblance was not significant because of the absence of degenerated collagen and the "chicken-wire" network of reticulum, considered important criteria of the Aschoff body.

A similar response, but much milder in degree, was observed in mice subjected to repeated intravenous injections of the same organism. Lesions simulating the Aschoff body were not found in these animals.

A study of the lesions produced by a strain of greening streptococci in rats and mice, correlated with reports of similar lesions in other animals, leads to the belief that the monocytic response in the rat, rabbit, and probably other animals following the injection of these microörganisms is merely the reaction of an immune or partially immune host to a pathogen of relatively low virulence and that the formation of giant cells, as well as structures superficially resembling Aschoff bodies, is a minor modification of this general reaction.

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DESCRIPTION OF PLATE

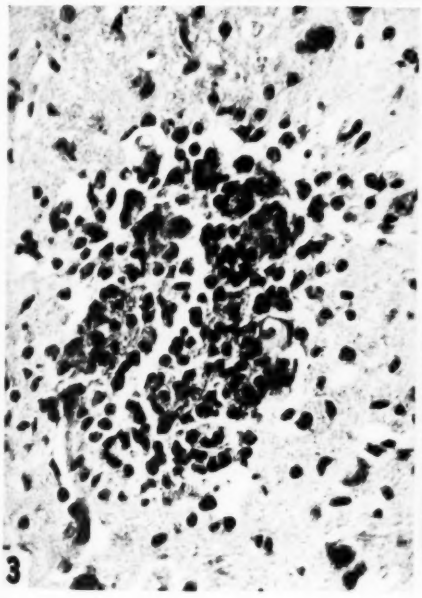
PLATE 72

FIG. 1. Myocardial lesion resembling an Aschoff body in a rat subjected to 8 intracardiac injections with a strain of greening streptococcus. The animal died 4 days after the last injection, but survived the first injection 18 days. Hematoxylin and eosin stain. × 500.

FIG. 2. Vegetations, polypoid in character, upon the mitral valve and the left auricular endocardium in a white rat given 17 intracardiac injections with greening streptococci. The animal survived the first injection 38 days and died 21 days following the last injection.

FIG. 3. Brain lesion consisting entirely of monocytes in a rat given 5 intracardiac injections with greening streptococci. The animal survived the first injection by 9 days and the last by 2 days. Hematoxylin and eosin stain. × 330.

FIG. 4. Healing, partial glomerular infarction in a rat given 9 intracardiac injections with greening streptococci. This animal survived the first injection by 14 days and the last by only 1 day. Hematoxylin and eosin stain. × 380.



Gross, Cooper and Phillips Response to Greening Streptococci

UNIV. OF MICH.

RETICULUM CELL SARCOMA OF LYMPH NODES*

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Is it justifiable to extend the term "reticulum cell sarcoma" for tumors of lymph nodes as widely as seems to be the tendency in present literature? We believe it is not, since the term cannot be proved to be either proper from the standpoint of general tumor nomenclature or useful from the anatomo-clinical point of view.

It is our hope that, by adopting sharp criteria for the diagnosis of reticulum cell sarcoma of lymph nodes, it will be possible in the future to study with some accuracy the prognosis and radiosensitivity of this type of lymphoid tumor. At present, owing to confusion in nomenclature, the data in medical literature are by no means clearly stated. In view of this growing tendency to use inexactly the term "reticulum cell sarcoma," once sharply defined but now broadened to include almost any lymphoid tumor with proliferation of reticular tissue regardless of degree, we have reëxamined a group of 710 lymphoid tumors † and have selected for study those fulfilling the more rigid initial criteria.

Soon after Aschoff's delineation of the reticulo-endothelial system as a physiological unit, attempts were made to describe special pathological entities of the system. Attention was focused on certain tumors whose constituent cells showed morphologic characteristics reminiscent of the histologic and physiologic properties of the reticulo-endothelial cells.

Goormaghtigh [1] was the first to describe a "malignant proliferation of the reticulo-endothelial tissue of lymph-nodes" made up of cells "different from the round mobile elements of the other benign or malignant lymphoid tumors," and proposed the name "réticulo-endothéliome." Because it implied a tumor of the reticulo-endothelial system as such, this term has met objections. Oberling [2] and Oberling and Raileanu [3] in France used the term "réticulosarcome," and Parker and Jackson [4] in this country popularized

* Received for publication October 17, 1940.

† These tumors were from the files of the Huntington Memorial, Peter Bent Brigham, New England Deaconess and Pondville Hospitals.

the term "reticulum cell sarcoma." The papers of Oberling so focused attention on the subject that Delbet [5] in France was led to question "whether there is not nowadays a sort of intoxication which leads one to see more reticulum cell sarcomas than there actually are." Soon afterwards, Roulet [6, 7] wrote his papers on "Retothelsarkom" of the lymph nodes. The frequency with which more and more reticulum cell sarcomas had been described (culminating in the long treatise of Pittaluga [8] who devoted a good part of his book, which appeared in 1934, to tumors of the reticulo-endothelial system) led Bianchi [9] to criticize this tendency. His article redelineating the classic concepts of histogenesis of lymphoid tumors is worth reading.

The classification of the Lymphatic Tumor Registry [10] defines the reticulum cell sarcoma as a malignant tumor of reticulocytes (monocytes) and implies that it is closely related to the sarcomatous form of Hodgkin's disease.

For the diagnosis of reticulum cell sarcomas we use the rather restricted criteria given by Oberling [2] in his original description of "réticulo-sarcome indifferencié." These tumors have the following characteristics: The new growth is formed of syncytial masses of an undivided or slightly fenestrated protoplasm whose limits are ill-defined and connected with zones of more or less differentiated lymphoid tissue. The fenestration, by exaggerating the internuclear spaces, may give a reticular structure. In these undivided masses of protoplasm there are many irregularly distributed oval or indented nuclei, with a well defined nuclear membrane, sparse powdery chromatin granules, and one or two prominent nucleoli. Not infrequently the nuclei give the impression of being empty because of the striking contrast of the darkly stained nuclear membrane and the scanty and lightly stained chromatin. These nuclear characteristics are helpful in differential diagnosis among many other types of malignant cells in which nuclear hyperchromatism is prominent. Some mitoses and occasionally some isolated tumor giant cells also may be found. Mitoses never appear in great numbers. It is not rare to see some lymphocytes scattered throughout the syncytium.

The silver methods bring out variable types of reticulum. These syncytial protoplasmic masses may show a dense network or only scattered, fine, fragmented threads of reticulum.

The neoplastic proliferation of reticulum cells presents some typical features that enable one to relate the new growth to the normal reticulum cells. Thus, the masses of undivided or reticulated syncytial cells reproduce the primitive character of the mesenchymal syncytium.[11-13] The nuclei keep their embryonal character, typical also of the reticulum cells.

One of the characters of reticulum cells more frequently quoted, and therefore ascribed to their new growths, is the property of forming argentaffine reticulum fibrils.[14-16] While this is true of these tumors, it is by no means pathognomonic of them.[17] The erroneous assumption that a new growth of lymph nodes showing reticulum proliferation must be classified as a reticulum cell sarcoma is the source of many misleading descriptions. Furthermore, one must not attach too much importance to the production of fibrillar reticulum, paradoxical as this statement may seem. Very undifferentiated reticulum cell sarcomas may show little reticulum within the syncytial masses (Figs. 1 and 2). On the other hand, we are all aware of the striking reticulum proliferation in Hodgkin's disease and in the stromal reaction of the common epidermoid carcinomas, not to speak of many other processes.

In other words, we think that while the presence of reticulum formation affirms the reticular nature of the syncytium, its absence or rarity does not exclude the diagnosis. In the absence of reticulum, special care must be taken to exclude the possibility of the tumor being metastatic from a highly undifferentiated carcinoma.

Other anatomical and physiological properties of the reticulo-endothelial system (phagocytosis, mobilization, lining of lymph sinuses) are not to be considered essential for diagnosis, for otherwise almost any tumor having a mesenchymal origin could be called a reticulum cell sarcoma. The primitive mesenchymal cell may present in its evolution almost any of the characteristics commonly referred to the reticulo-endothelial system. Not only must the reticulum cell sarcoma be differentiated from mesenchymal tumors in general, but from other lymphoid tumors as well. In distinguishing it from tumors of the latter group, we place the dividing line at the stage where appreciable lymphopoiesis can be detected.

Thus we regard the reticulum cell sarcoma as the most undif-

ferentiated form of the lymphoid tumors according to this schema:

- Sarcomas of lymphoid tissue
  - Reticulum cell type
    - Undifferentiated or syncytial
    - Reticular
  - Mixed type
    - Combination of reticulum and lymphoid cells
  - Lymphoblastic type
    - Lymphoblastoma or lymphosarcoma
  - Lymphocytic type
    - Malignant lymphoma

We have attempted to diagram this relationship in Figure 3, which is synthesized from 5 cases representing each of the types of tumor included in the classification.

The assumption that these tumors are best considered as endotheliomas because they have been seen to arise from the lining cells of the lymph sinuses can be rejected. It is generally accepted that both the lining cells of the sinuses and the reticulum cells of the pulp or germinal centers are embryologically, morphologically and physiologically the same element.[18] If tumors of this sort were derived only from the endothelial cells of lymph sinuses they would not appear in closed lymphoid formations such as the tonsil. One of our specimens (No. 5 in Summary of Cases) comes from a tonsil.

Our concept of the normal lymphoid tissue is a network of undifferentiated cellular reticulum (stem tissue) in whose interstices lie lymphoid cells (lymphoblasts, prolymphocytes, lymphocytes), the offspring of the former. Thus, the complex of *reticulum cells-lymphoid cells* cannot be divorced without destroying the concept of the essential unity of the lymphoid tissue. Any malignant proliferation of lymphoid tissue must then be composed not only of lymphoid cells alone (as is sometimes assumed) but also of reticulum cells. Since the cellular reticulum is the vital constituent, being the stem tissue rather than a simple stroma of the normal lymphoid structure, a tumor of the lymph-

oid cells does not contain reticulum cells only as a supporting element (stroma), but as a part of the tumor itself. These reticulum cells may be either survivors of the original lymph node structure or active participants in the tumor.

The sarcomas of lymphoid tissue can best be classified by the predominant cell type. Malignant proliferation of lymphoid cells of varying degrees of differentiation may occur. These may present different features, although closely related by their histogenesis. This genetic relation to one another should not be permitted to impair the utility of a histologic differentiation, although they cannot be sharply divided. Figure 3 shows their relationships.*

With regard to the structure of the common lymphosarcoma, it is important to remember that in describing it Kundrat [19] first, and Paltauf,[20] Sternberg, [21-23] Banti [24] and Aresu and Scalabrino [25] afterward, mentioned the presence of both lymphoid cell and reticular cell proliferation. A particularly clear drawing in Ziegler's [26] textbook is eloquent enough. We believe that the statement by Goormaghtigh,[1] that the tumors he described were made up of cells different from those which constitute benign and malignant proliferations of lymph nodes, may have led to the erroneous idea that the lymphosarcomas were composed of nothing but lymphoid cells. The rediscovery that reticulum cells are often present in lymphosarcomas has, on the other hand, led to overemphasis of the reticulum cell sarcoma.

Of a series of 710 lymph node tumors examined in this study, 402 were discarded for various reasons. Many were cases of Hodgkin's disease. The rest, 308, were lymphoid tumors suitable for study. Both lymphatic leukemias with lymph node involvement and leukosarcomas were included. Among these we have been able to find but 11 examples of reticulum cell sarcoma, an incidence of 3.6 per cent, much less than is often reported. (See Summary of Cases.) This is due to the restricted use we make of this term.

This conception of the reticulum cell sarcoma is not original with us, nor has the nomenclature been modified by us. The tumor we recognize as a syncytial reticulum cell sarcoma was

* We have omitted Hodgkin's disease which we consider a more complicated process and still controversial.

observed and described long ago, albeit at times under various names ("Synzytium-Endotheliom"—Ciaccio;[27] "réticulo-endothéliome"—Goormaghtigh;[1] "réticulosarcome"—Oberling;[2] "Retothelsarkom unreife Form"—Roulet [6]).

APPENDIX

Summary of Cases

1. Female, 31 years of age. No. 40–1704.
   Initial location: neck.
   First symptoms developed January 1940.
   No follow-up.
   Histology: reticulum cell sarcoma (undifferentiated type).

2. Male, 40 years of age. No. 24–1590.
   Initial location: neck.
   First symptoms developed August 1924.
   No follow-up.
   Histology: reticulum cell sarcoma (undifferentiated type).

3. Male, 30 years of age. No. 40–1768.
   Initial location: neck. Mass in left supraclavicular fossa. Some shotty nodes in axilla and groins.
   First symptoms developed July 1923.
   Death: October 20, 1923.
   Duration: 3 months.
   Histology: reticulum cell sarcoma (zones of undifferentiated type).

4. Female, 68 years of age. No. 32–1137.
   Initial location: groin.
   First symptoms developed January 1932.
   Death: January 13, 1935.
   Duration: 3 years.
   Histology: reticulum cell sarcoma (undifferentiated type).

5. Female, 50 years of age. No. 37–1240.
   Initial location: tonsil.
   First symptoms developed May 1935.
   Patient alive and well March 30, 1940.
   Histology: reticulum cell sarcoma showing some differentiation (many lymphoblastic cells).

6. Female, 72 years of age. No. 36–311.
   Initial location: neck.
   First symptoms developed January 1936.
   No follow-up.
   Histology: reticulum cell sarcoma, somewhat more reticulated type (periphery well preserved).

7. Male, 17 years of age. No. 38–1644.
   Initial location: left axilla. (Widespreading to neck, axillary, inguinal and mediastinal nodes.)
   First symptoms developed April 1938.

Death: February 2, 1939.
Duration: 10 months.
Histology: reticulum cell sarcoma (with zones of undifferentiated type).

8. Male, 44 years of age. No. 36–1967.
Initial location: auricular gland (widespreading).
First symptoms developed in 1936.
Death: 1937.
Duration: 1 year.
Histology: reticulum cell sarcoma (somewhat differentiated zones of reticulated type).

9. Female, 54 years of age. No. 24–654.
Initial location: neck.
First symptoms developed April 1924.
No follow-up.
Histology: reticulum cell sarcoma (reticular type).

10. Male, 63 years of age. No. 30–1223.
Initial location: neck. (Extended to shoulder girdle and chest wall.)
First symptoms developed June 1929.
Death: May 17, 1930.
Duration: 11 months.
Histology: reticulum cell sarcoma (with zones of reticulated and undifferentiated types).

11. Male, 37 years of age. No. 24–204.
Initial location: neck (widespread adenopathy).
First symptoms developed January 1923.
Death: May 25, 1924.
Duration: 1½ years.
Histology: reticulum cell sarcoma (differentiated type).

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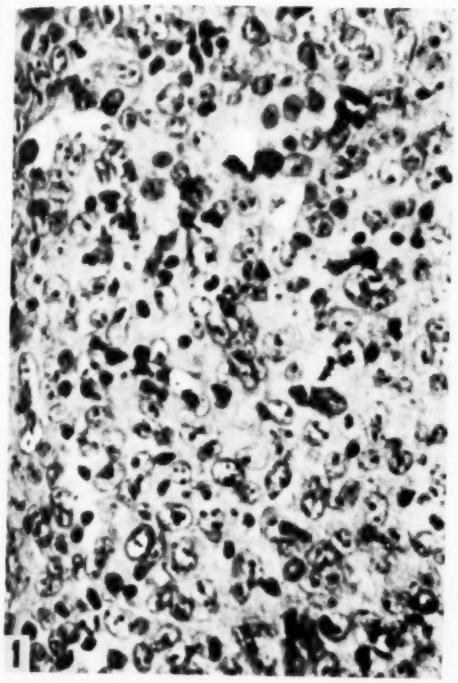
DESCRIPTION OF PLATES

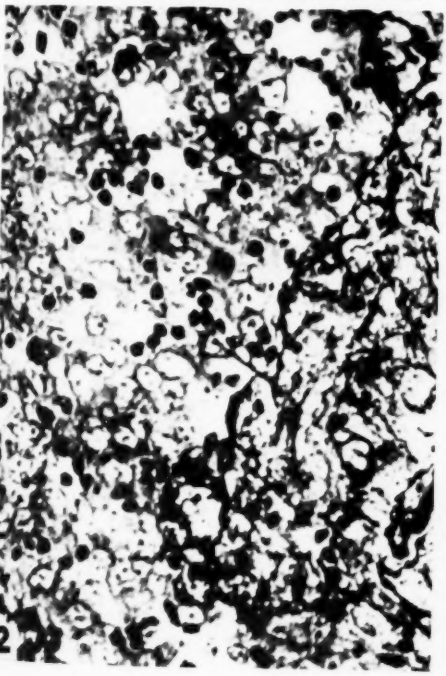
PLATE 73

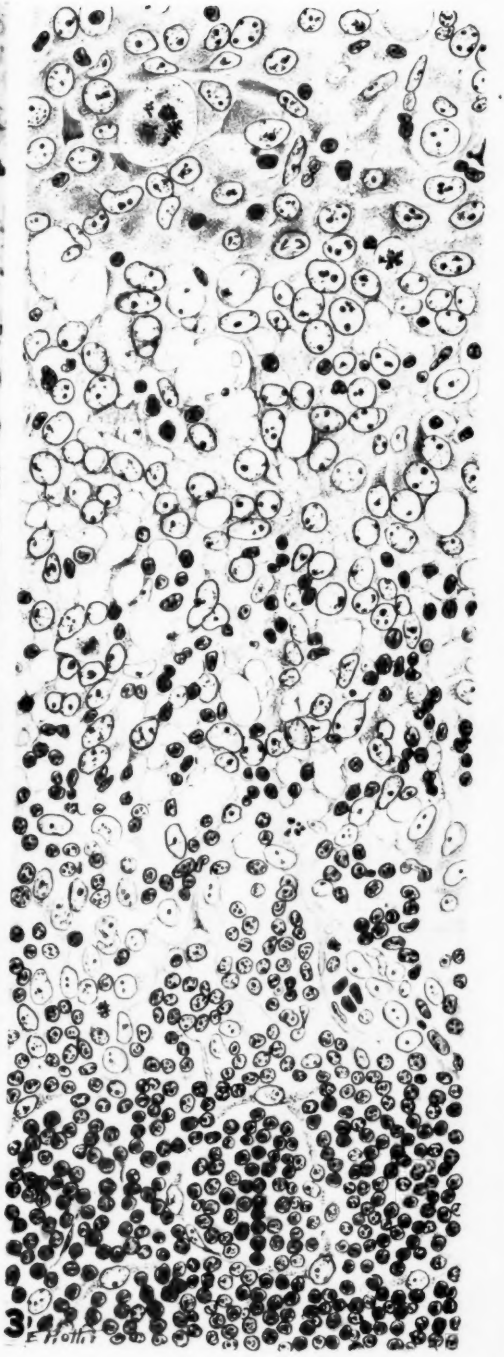
FIG. 1. Reticulum cell sarcoma, showing syncytium. × 410.

FIG. 2. Reticulum cell sarcoma (case 1), showing syncytium and reticulum. × 410.

FIG. 3. Diagrammatic drawing taken from actual cases, showing changes in degree of differentiation from reticulum cell sarcoma of syncytial type to malignant lymphoma.







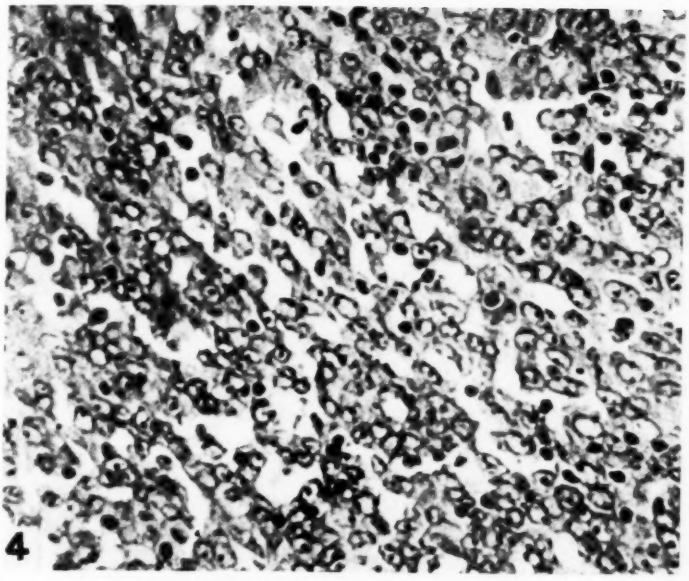
Warren and Picena

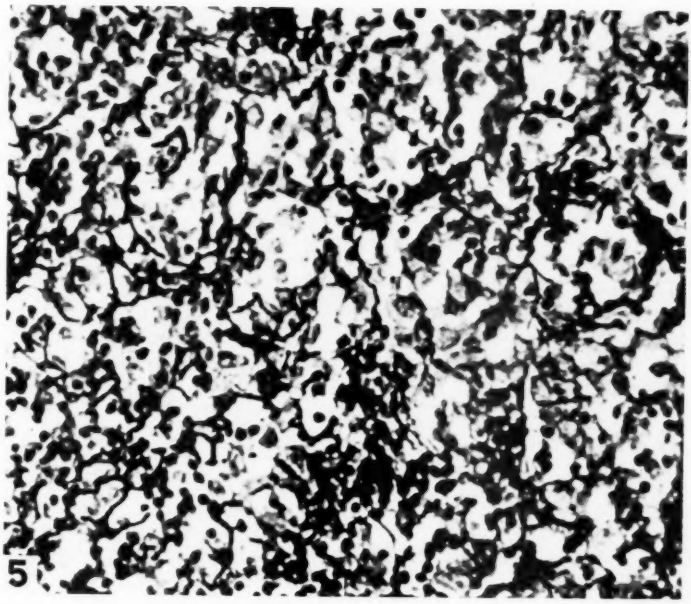
Reticulum Cell Sarcoma of Lymph Nodes

PLATE 74

FIG. 4. Reticulum cell sarcoma (case 9), reticulated type. × 410.

FIG. 5. Reticulum cell sarcoma (case 9), reticulated type, stained for reticulum. × 410.





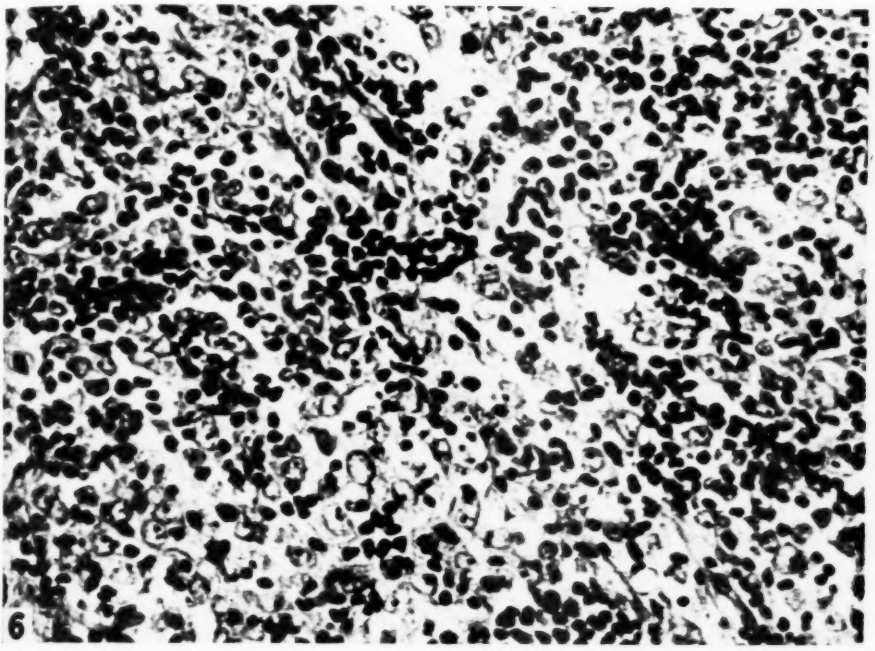
Warren and Picena Reticulum Cell Sarcoma of Lymph Nodes

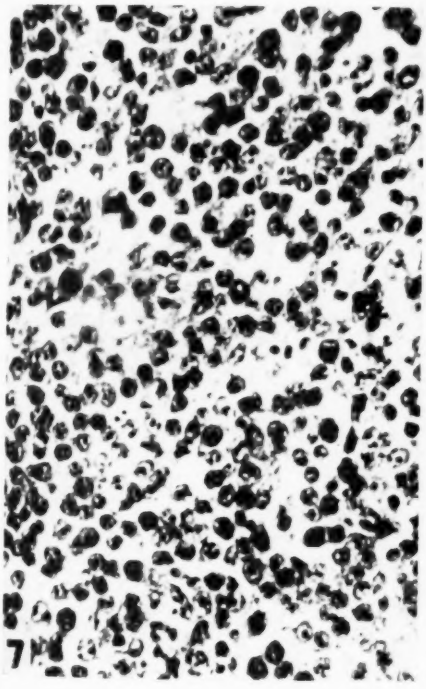
PLATE 75

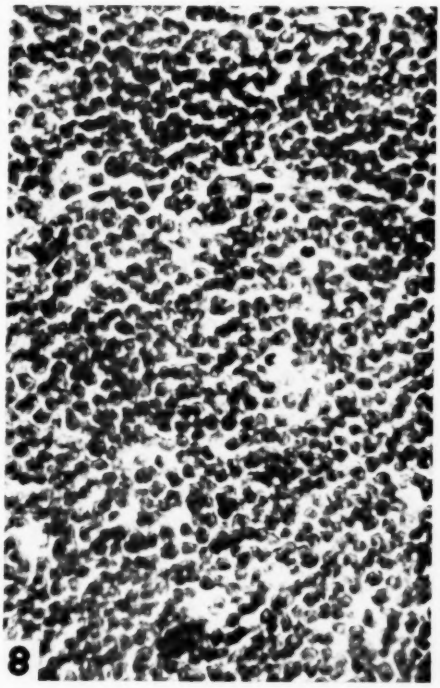
FIG. 6. Reticulum cell sarcoma (case 9), mixed type. × 410.

FIG. 7. Lymphosarcoma (lymphoblastoma type). No. 32–1563. × 410.

FIG. 8. Malignant lymphoma. No. 39–73. × 410.







Warren and Picena

Reticulum Cell Sarcoma of Lymph Nodes

UNIV. OF MICH.

OBSERVATIONS WITH DIFFERENTIAL STAINS ON HUMAN ISLETS OF LANGERHANS*

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Whereas reports on the number and size of the islets of Langerhans under normal, experimental and pathological conditions, and on changes observable with routine stains, would fill volumes, there is very scant literature available on observations made with the aid of specific stains, especially in respect to human material. This is due to the fact that up to 1931 the only known differential stains for the study of islet cells were the neutral stains of the type Lane and Bensley described. These stains give beautiful pictures on guinea pig material. However, in other animal species and on human material their results are much poorer and often very hard to interpret. The use of the Mallory-Heidenhain azan stain as a differential stain for islet cells was first suggested by Bloom in 1931. It can be used successfully in practically all species, but it does not give a clear picture of the beta cells which, according to most authors, must be considered the source of insulin. This may explain the fact that, as Warren stated, no attempt has been made so far to do differential counts on human islets. In 1939 I published a staining method that gives a sharp differentiation between alpha and beta cells in all species. Another specific stain for dog material was developed by Richardson in 1940.

The purpose of this paper is to give an account of observations made with specific stains on 70 human pancreases, normal and pathological.

Material and Methods

Most of the material came from autopsies at the Billings Hospital of the University of Chicago. Only those cases in which autopsy was performed within 4 hours after death are included in this study, although in many instances excellent results were

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obtained with the author's chromium hematoxylin-phloxin stain as late as 10 hours after death. Two islet cell adenomas were obtained from the Department of Surgery of the same hospital. Five pancreases from diabetic patients were received from the Laboratory of Pathology of the New England Deaconess Hospital in Boston. The total number of pancreases from diabetic patients was 11. The remaining 59 were from nondiabetic individuals. In this last group there were 3 with gross pancreatic lesions: benign islet cell adenoma with hypoglycemia, 2 cases; islet cell carcinoma with hypoglycemia, 1 case.

One other, rather unique, case (No. 4561) will be mentioned here because the clinical picture was that of an insulin-producing tumor of the pancreas. A man, aged 56 years, complained of dizzy spells and repeated fainting for 4 months before admission. During his stay in the hospital typical hypoglycemic attacks were observed with blood sugar values as low as 31 mg. per 100 cc. At laparotomy the pancreas was found to be normal. Radon seeds were inserted and the abdomen was closed. The patient died of shock on the next day. At autopsy the pancreas contained no neoplasm nor was there any hyperplasia of the islets present. A huge, ovoid, solid tumor, bulging from the upper aspect of the right lobe of the liver and extending deeply into the liver itself, was the only essential finding at autopsy. On microscopic examination the tumor proved to be a fibroma. Bio-assay failed to show the presence of insulin in it. The mechanism of hypoglycemia in this case is not well understood.

Of all material, slices not exceeding 2 mm. in thickness were cut and fixed in Bouin's fluid, in Bayley's modified Zenker-formaldehyde and in Stieve's fluid. After 30 minutes, when the slices were firm enough, they were halved in thickness with a razor blade and replaced in the fixing fluid for 24 hours. All fixatives proved to be quite satisfactory for the specific stains, but Bouin's fluid was found to be the best. The modified Bouin's fluid as described in the original chromium hematoxylin-phloxin technic, though it gives the sharpest differentiation of the beta cells, was not used because its penetration is rather slow, and blocks often are unevenly fixed. Fixation at ice box temperature proved of no advantage. The slices were embedded in paraffin and cut at from 2 to 4 μ.

As neither the neutral stains nor Richardson's technic gave satisfactory results, only the following stains were used:

1. The Mallory-Heidenhain azan stain. It was found that the D cells will stain far sharper and darker if the staining time with azocarmine is prolonged. Optimal results were obtained by staining for 10 to 16 hours at 65° C. in a 0.05 per cent azocarmine G solution containing 1 per cent acetic acid. Differentiation in aniline-alcohol may take a rather long time after this prolonged staining, but it may be greatly shortened by adding about 10 per cent of distilled water to the differentiating fluid. Alpha cells can be brought out with extreme clarity if the sections are treated before staining for about 30 seconds with a 0.1 per cent solution of potassium permanganate and subsequently decolorized with sodium bisulfite.

2. Mallory's phosphotungstic acid hematoxylin. If after permanganate oxidation the sections are mordanted for about 1 hour or longer in a 2 to 5 per cent solution of iron alum and then stained in Mallory's phosphotungstic acid hematoxylin for 8 to 24 hours, depending on the type of fixation and on the thickness of the sections, very sharp pictures are obtained with excellent visualization of certain cellular details. Zymogen granules are a rusty red, alpha granules a deep steel gray, mitochondria and terminal bars blue-black. Beta granules are unstained and D granules are either unstained or a dull lilac. This stain is also recommended for the staining of other structures, such as cross striations in muscle tissue and epithelial fibers.

3. Gomori's chromium hematoxylin-phloxin stain. Since the original publication of this technic, attempts have been made to simplify the method and, at the same time, to make it work with a high degree of constancy of results. It was found that after a suitable pretreatment (refixation and permanganate oxidation) practically any basic stain will bring out the beta cells more or less selectively. However, only acridine red of Grübler (but not other brands of the same dye) and chromium hematoxylin were found to give perfectly clear definition. As the counterstaining of sections stained with acridine red is somewhat difficult, it is better to depend on hematoxylin. The following modifications of the technic proved to be useful: (a) Bouin's fluid is entirely satisfactory for refixation; (b) the amount of potassium dichromate

in the hematoxylin solution has been decreased because with the original amount of dichromate the stain will keep its full staining power and selectivity for only about 10 days; (c) for the better staining of the alpha granules a thorough washing of the sections after phosphotungstic acid is recommended. The summary of the improved method is as follows:

1. Run sections through xylol and alcohols to water.
2. Refix in Bouin's fluid for from 12 to 24 hours.
3. Wash sections thoroughly in tap water to remove picric acid.
4. Treat sections for about 1 minute with a solution containing about 0.3 per cent each of potassium permanganate and sulfuric acid.
5. Decolorize with a 2 to 5 per cent solution of sodium bisulfite. Wash.
6. Stain in the following hematoxylin solution under microscopic control until the beta cells stand out deep blue (about 10 to 15 minutes): Mix equal parts of 1 per cent aqueous solution of hematoxylin (National Aniline hematoxylin C. P. and Grübler's dark hematoxylin are very satisfactory) and of 3 per cent solution of chromium alum. Add to each 100 cc. of the mixture 2 cc. of 5 per cent solution of potassium dichromate and 2 cc. of N/2 (about 2.5 per cent) sulfuric acid. The mixture is ripe after 48 hours and can be used as long as a film with a metallic luster will continue to form on its surface after 1 day's standing in a Coplin jar (about 4 to 8 weeks). Filter before use.
7. Differentiate in 1 per cent hydrochloric acid-alcohol for about 1 minute.
8. Wash under the tap until the section is a clear blue.
9. Counterstain with 0.5 per cent aqueous solution of phloxin for 5 minutes. Rinse.
10. Immerse in 5 per cent phosphotungstic acid solution for 1 minute.
11. Wash under the tap for 5 minutes. The section should regain its red color.
12. Differentiate in 95 per cent alcohol. If the section is too red and the alpha cells do not stand out clearly enough, rinse the section for about 15 to 20 seconds in 80 per cent alcohol.
13. Transfer to absolute alcohol, clear in xylol and mount in balsam.

The results of the three staining methods were compared by staining each of three consecutive serial sections cut at 2 μ with a different stain. The methods were found to be completely comparable, and no overlapping of alpha and beta cells with different stains was observed. With chromium hematoxylin-phloxin the beta cells are blue, alpha cells red, D cells from pink to red and indistinguishable from alphas. With the azan stain the beta cells have indistinct dingy grayish granules, the alpha cells ruby red granules and the D cells deep blue granules. Phosphotungstic acid hematoxylin does not stain the beta granules; the alpha granules are steel gray to blue-black; the D granules are either unstained

or a pale lilac. In general, neither the azan stain nor phosphotungstic acid hematoxylin will yield clear pictures if the material is older than 2 to 3 hours, whereas, as mentioned, the chromium hematoxylin-phloxin stain may give beautiful pictures even if the material is older.

Observations

Differential Counts on the Islets Under Normal and Pathological Conditions

Sections of each pancreas were stained with chromium hematoxylin and the Mallory-Heidenhain azan stain. Of 70 pancreases, the state of preservation in 6 was too poor for any differential stain. The azan stain was unsatisfactory in 9 more. The remaining 55 were satisfactory for complete differential counts. At least 6 islets were counted in every section, totalling from 500 to 1200 cells.

Although in some pancreases the proportion between the numbers of the different cell types is rather uniform throughout all islets, usually, even under normal conditions, the ratio is extremely variable not only in different areas, but also in the same section.* It is by no means uncommon to find an islet with a beta:alpha ratio of 6 or higher next to another islet having a ratio far below 1, *i.e.*, consisting predominantly of alpha cells (Fig. 1). Since the alpha cells have a tendency to nestle against the walls of capillaries at the periphery of the cell cords, whereas the beta cells most often occupy the more central, avascular areas of the cell cords(Fig. 2), it is a common observation that high vascularization of an islet means a high alpha count in that islet. Islets having many capillaries may consist almost exclusively of alpha cells. On the average, around 60 to 90 per cent of all islet cells are beta cells, the rest being alpha cells and D cells. In approximately 66 per cent of all cases without demonstrable pathology in the pancreas, the beta:alpha ratio was between 3 and 8; in 15 per cent between 1.2 and 3; in 19 per cent between 8 and 11. These counts having been done with the chromium hematoxylin-phloxin stain, D cells were counted as alpha cells.

The azan stain shows that the D cells are the least numerous

* The same thing was found to be true in respect to the cellular composition of the islets of the dog. This is in contrast with the findings of Hunt who found the beta:alpha:D ratio to be fixed at 75:20:5.

of the three cell types. Their number is roughly proportionate to that of the alpha cells. Under normal conditions they account for from 2 to 8 per cent of all islet cells. Their predilectional sites are exactly the same as those of the alpha cells. The same applies to their shape and size as well as to the size and distribution of their granules, the only difference being the special staining reaction of the D granules. Moreover, transitions between alpha and D cells are often observed. All intermediate shades from ruby red to clear blue granules can be seen in any adequately stained section of fresh, well fixed material. There may be serious difficulty in telling whether an occasional cell is more like an alpha cell or a D cell. There is some evidence, as it will be pointed out later, that D cells represent but a stage of ageing of the alpha cells.

These wide variations in the cellular composition of the islets under normal conditions cast considerable doubt on the value of reports dealing with the increase or decrease in the numbers of either beta or alpha cells under various experimental conditions.

There is great variability also in regard to the extent and degree to which the cells are granulated. Sometimes 80 to 90 per cent of all beta cells are densely packed with dustlike, dark blue granules. In most cases, however, granulation is less complete. Three different forms of degranulation of beta cells have been observed, which often occur in combination:

1. Diffuse degranulation (Fig. 3). Throughout the islet the beta granules show rarefication. They are strewn more or less sparsely but uniformly in the cytoplasm of the cells.

2. Discontinuous degranulation (Fig. 4). Many of the beta cells in an islet are completely, or almost completely, degranulated; while others, scattered at random, are fully granulated and dark.

3. Margination (Fig. 5). Granules can be seen in the cell cords only at their walls adjoining capillaries. They occupy one side of the cell only. The central parts of the cell cords are degranulated. Under low power the lobules of the islet are pale areas, rimmed with dark blue.

Both diffuse and discontinuous degranulation has been found, though less commonly, in alpha cells also. Considerable discontinuous degranulation of both beta and alpha cells may render an

exact differential count impossible, because the cells have lost their distinctive characteristics. This was the case in six instances in the present series.

No observations have been made on the degranulation of D cells.

In diabetic patients the beta:alpha ratio was found often to be lower than in nondiabetics, but the small number of cases does not permit drawing any sweeping conclusions. Out of 7 cases in which it was possible to do complete counts, 4 had very low beta: alpha ratios (0.74, 0.76, 0.83 and 1.4). The rest were entirely within the limits of the normal average. The beta cells, which are preserved even in greatly shrunken, hyaline islets (Fig. 6), often are remarkably dark and densely crowded with granules. On account of the blue-staining masses of hyalin it is sometimes hard to do differential counts for D cells in azan-stained sections, but whenever it was possible (6 cases), the D cells were found to be present in normal numbers.

In three cases of *pancreatic tumor with hypoglycemia* the findings were as follows:

Case No. 4838 (benign adenoma). Pancreatic tissue, adherent to and resected with the tumor, as well as autopsy material, was examined. However, the latter was too poorly preserved for cytological studies. In the surgical specimen both beta and alpha cells were extremely degranulated and pale (Fig. 7). The beta: alpha ratio was 8. No D cells were found.

In case No. U233821 (benign adenoma), extensive resection of the pancreas having been performed, it was possible to examine material from several parts of the gland. The islets presented extreme diffuse degranulation of both beta and alpha cells. The beta:alpha ratio was 4. Not a single D cell could be found. In addition, many of the islets showed very marked hydropic changes in the beta cells, of exactly the type that is considered the most characteristic diabetic change of the pancreas (Allen, Martin). The interesting thing was that no islets with moderate changes were found. Either they were not hydropic at all or very markedly so (Fig. 8). The hydropic degeneration of the beta cells may have been due to overburdening of the islets by the large amounts of intravenous dextrose required for the control of hypoglycemic seizures.

In case No. 4449 (carcinoma with metastases) the islets were found to be very small, poorly demarcated and the cells greatly shrunken (Fig. 9). Some of the cells could be identified as beta or alpha cells, but the overwhelming majority took an indifferent purplish stain, without recognizable granulations. In azan-stained sections a few pale blue cells were tentatively identified as D cells.

In one case (No. 4561) of *tumor of the liver with hypoglycemia,* the findings were very interesting. The beta:alpha ratio was as low as 1.13 and 22 per cent of all cells were D cells. This is the highest D ratio observed in the whole series. In some of the islets practically all of the peripheral cells were D cells (Fig. 10). Both beta and alpha cells were densely granulated.

The Acino-Insular Relationship

The acino-insular relationship has been debated practically ever since it became known that islets are of a tissue distinctly different from the acini. There are champions of the fixed nature and noninterchangeability of the two tissues once they are fully differentiated (Lane, Bensley, Ukai, Homans, Allen and others), whereas a numerous group (Saguchi, Vincent, Otani, Sergeyeva and others) claim that the relation between the acini and islets is dynamic rather than static, and that transitions from acini to islets and *vice versa* occur under functional stimuli even in adults. The specificity of the cell types is another moot point. Bensley does not believe in the possibility of transformation between cell types alpha and beta. According to Bloom the D cells possibly represent but a stage in the evolution of beta cells.

The main source of confusion is a cell type first described and erroneously interpreted as a form of transition between acinar and islet cells by Mankowski in 1902. Under normal circumstances they are present in very small numbers, but under certain pathological and experimental conditions, such as obstruction of the pancreatic ducts by neoplasms, inflammation or ligation; long-continued feeding of animals with dry fodder, and feeding with cream, they may appear in large numbers. They develop from acinar cells, and all stages of transition between a normal, zymogen-laden acinus cell and a full-fledged Mankowski cell can be observed. The typical Mankowski cell contains a rather fine, acidophilic granulation and in many respects is similar to an alpha

cell. However, as Bensley remarked, their granules are not true alpha granules. They differ from alpha granules in that they are coarser, much less uniform in size and are never as densely packed as the latter. (This is shown in Figs. 11 and 12, reproduced by the courtesy of Sylvia H. Bensley.) It may be added that their staining reactions are also sufficiently different from those of the alpha granules to permit easy discrimination. With the neutral stains and with chromium hematoxylin-phloxin they stain exactly like alpha granules and also with phosphotungstic acid hematoxylin, provided the section was not, or not sufficiently, oxidized with permanganate before staining. However, after energetic permanganate treatment the Mankowski granules will stain exactly like zymogen granules, *i.e.*, rusty red, while alpha granules remain deep steel gray. In a well differentiated azan-stained section the alpha granules are distinctly red, whereas the Mankowski granules are orange red. The color contrast can be greatly enhanced by permanganate pretreatment, as suggested by me. In permanganate-treated azan-stained sections the alpha granules are ruby red, while the Mankowski granules are orange yellow. Transitions between Mankowski cells and alpha cells are never observed. The former go on to degeneration by vacuolization or homogenization of their cytoplasm and possibly are the precursors of the "granular cells of the acini" described by Bayley. The Mankowski cells are probably the result of some pathological interference with the secretion and discharge of zymogen.

One of the commonest findings is that of the presence of alpha cells in the acini (Fig. 13). Occasionally, also, D cells can be found at the same site (Fig. 14). Both kinds of cells conform perfectly in regard to shape, size and orientation with the cells of the acini. Their presence cannot be suspected without special stains. Although small groups of beta cells are often seen scattered throughout the acinar parenchyma, they practically never blend so perfectly with the acinar cells as do alpha or D cells. They always give the impression of being foreign bodies wedged in between individual acini.

Alpha cells are often observed also among the cells lining small ducts and in solid epithelial buds continuous with duct epithelium (Fig. 15). Most often their granules stain a very clear ruby red, but in some of these cells the granules will stain purplish

and occasionally even bluish. Cells in the ducts, containing clear blue granulations and indistinguishable from D cells (not to be confused with blue-staining goblet cells), were observed very rarely in this series. The conspicuously low D:alpha ratio among duct cells and in young islets as compared to that found in older islets detached from the ducts suggests the possibility that D cells are nothing but aged alpha cells. No transitions between beta cells and any other cell type found in the pancreas could be observed.

In summarizing the above data it may be said that alpha cells develop from duct epithelium; D cells are probably aged alpha cells; the origin of beta cells is unknown. There is no evidence of transformation either between acini and islets or between the cell types alpha and beta, or D and beta.

Cytological Character of Tumors Causing Hypoglycemia

Two benign adenomas and one carcinoma were observed in this series. The histological structure of these tumors will not be described in detail here. The adenomas belonged to the usual highly vascularized, trabecular type; the carcinoma was a solid, rather anaplastic tumor which at some places showed abortive gland formation. With special stains no well defined cell types, as found in normal islets, could be demonstrated in any of them. The cells took an indifferent shade with all stains and contained no identifiable granules. In case No. 4838 the tumor cells showed extremely fine, dark gray, dustlike granules, some of which were rod-shaped, with phosphotungstic acid hematoxylin. Their morphology, however, was entirely different from that of normal alpha granules, being much closer to that of mitochondria.

Other Changes in the Islets Under Various Conditions

Vacuolization of beta cells was observed in 5 of the nondiabetic cases (Fig. 16). Hydropic changes were seen in the beta cells in one case of islet cell adenoma, as already mentioned. None of the pancreases from diabetic patients showed hydropic degeneration of the beta cells.

Moderate hyalinosis of the islets was observed in 2 nondiabetic patients, one dying at the age of 47 years of a pneumococcic meningitis, the other at the age of 55 years of renal insufficiency. Both had also hyaline changes in the small arteries.

SUMMARY

The cellular composition of the pancreatic islets in normal human material shows a wide variation. On the average, the beta:alpha ratio varies from 3:1 to 8:1. D cells constitute about 2 to 8 per cent of all islet cells.

Both alpha and D cells are found in relationship to the acini as well as in the epithelium of ducts. No transitions between acini and islets, or between the cell types beta and alpha, have been observed. All intermediate stages between alpha and D cells can be demonstrated.

It was impossible to identify the cells of three tumors causing hypoglycemia as of any of the normally occurring types.

Hyalinosis of the islets was present in 2 nondiabetic cases.

NOTE: The author wishes to express his appreciation to Shields Warren of the New England Deaconess Hospital, Boston, and to the members of the Department of Pathology of the University of Chicago for their kind collaboration and help in obtaining fresh material; also to L. R. Dragstedt and A. Brunschwig of the Department of Surgery of the University of Chicago for their permission to use their cases.

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DESCRIPTION OF PLATES

PLATE 76

All figures on this plate were stained with the chromium hematoxylin-phloxin method and photomicrographs were taken with an orange-yellow filter.

FIG. 1. Two adjoining islets, the one at the left with a beta:alpha ratio of 10 (partly marginated); the other with a beta:alpha ratio of 0.9. × 160.

FIG. 2. Typical arrangement of cell types. Light alpha cells line the periphery of the cell cords and dark beta cells occupy the more avascular central areas. × 160.

FIG. 3. Diffuse degranulation. × 320.

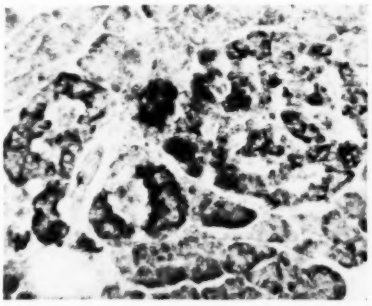
FIG. 4. Discontinuous degranulation with scattered dark beta cells. × 320.

FIG. 5. Margination. × 260.

FIG. 6. Hyaline islet with dark beta cells from a patient with diabetes. × 260.

FIG. 7. An islet from case No. 4838. × 260.

FIG. 8. Hydropic degeneration of beta cells in an islet from case No. U233821. × 520.



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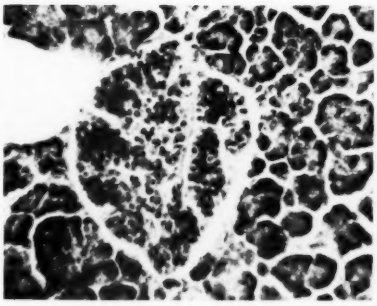

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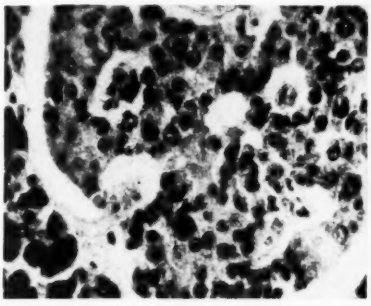

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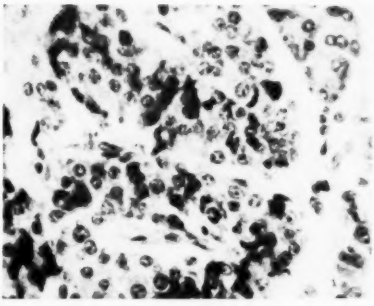

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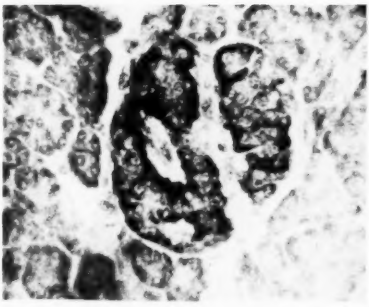

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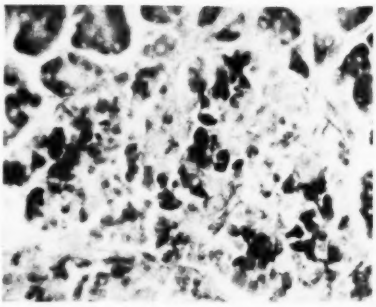

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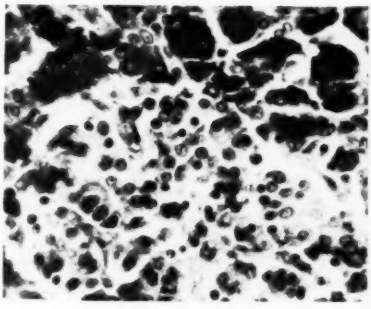

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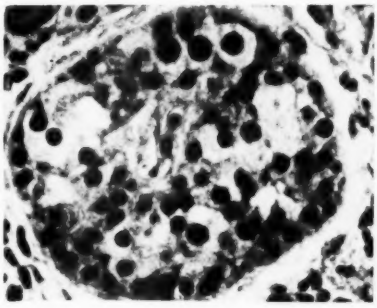


8

Gomori

Human Islets of Langerhans

Plate 77

Figures 9 and 16 were stained with the chromium hematoxylin-phloxin method; Figures 10 and 14 were stained with the Mallory-Heidenhain azan method; Figures 11, 12, 13 and 15 were stained with the phosphotungstic acid hematoxylin method. Photomicrographs were taken with an orange-yellow filter.

Fig. 9. An islet from case No. 4449. × 260.

Fig. 10. An islet from case No. 4561. Most of the peripheral cells are D cells (dark). × 320.

Fig. 11. Alpha cells in an islet of a guinea pig. At this magnification the alpha granules do not show up very distinctly. × 800.

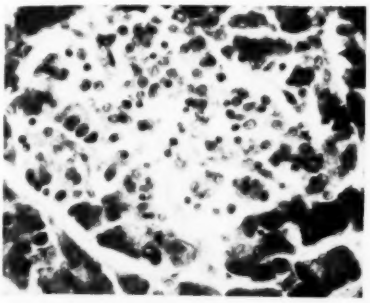
Fig. 12. Mankowski cells in the acini of the same guinea pig. Mankowski granules, although much smaller than those of zymogen, show up very distinctly at this magnification. × 800.

Fig. 13. Alpha cells in an acinus. To the left, the periphery of an islet. × 800.

Fig. 14. A D cell in an acinus. × 800.

Fig. 15. Alpha cells in ducts. × 520.

Fig. 16. Vacuolar changes in beta cells in the pancreas of a nondiabetic patient. × 800.



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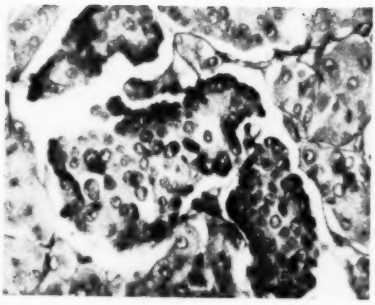

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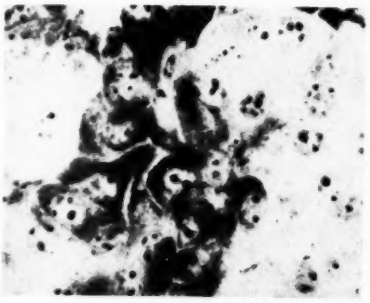

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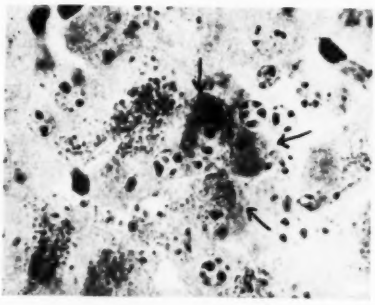

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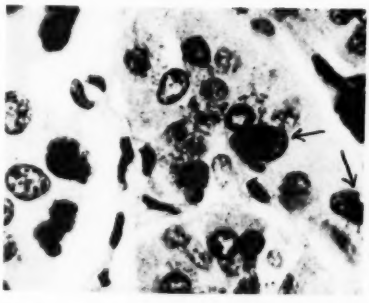

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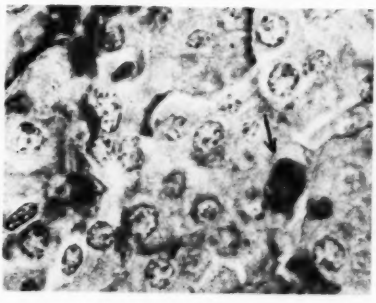

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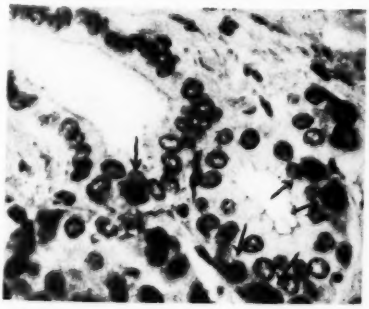

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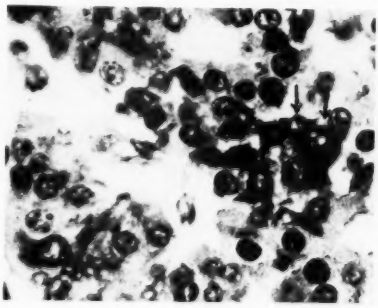

16

Gomori

Human Islets of Langerhans

EQUINE ENCEPHALOMYELITIS IN MAN*

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In a previously published review [1] of 100 cases in which the diagnosis of encephalitis had been made at the Boston City Hospital between the years 1928 and 1938, one unusual case was encountered. The pathological picture in this patient, who died in 1936, was so strikingly similar to that seen in the epidemic of eastern equine encephalomyelitis which occurred in the summer of 1938 in Massachusetts,[2,3] that it was considered advisable to report this case in detail.

The pathological picture of equine encephalomyelitis in man is so typical that it is thought to be specific.[4] The outstanding features are an extensive inflammatory reaction, with polymorphonuclear leukocytes in the perivascular spaces and diffusely throughout the nerve tissue, and destruction of the ganglion cells, as shown by severe degenerative changes in these cells with neuronophagia. The polymorphonuclear leukocytic inflammatory reaction is present even in those patients who die several or many days after the onset of symptoms. This differentiates the pathological picture from that of encephalitis lethargica, postinfectious encephalitis, acute anterior poliomyelitis, and the St. Louis type of encephalitis, in which diseases a few polymorphonuclear leukocytes may be present in the early stages of the disease but are rapidly replaced by lymphocytes and glial cells.

REPORT OF CASE

The patient was a white male, 23 years old, who died in April 1936 after an illness of 21 days. He entered the Boston City Hospital with vague complaints of chills and pains of 11 days' duration. Bronchopneumonia was suspected and X-rays of his lungs showed a patch of parenchymal infiltration at the base of the right lung. Pneumococci, type VII, were cultured from the sputum. Two days after entry the patient complained of severe headaches. The significant findings on neurologic examination were stiffness of the neck, right hemiparesis with Babinski sign and bilateral ankle clonus. The temperature ranged around 100° F. The spinal fluid, taken 5, 4,

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and 3 days before death, had, in all instances, polymorphonuclear leukocytic pleocytosis and an increase in the protein content. For instance, the spinal fluid taken 3 days before death was described as follows: The pressure was 190 mm. of water; the fluid was cloudy and contained 1,873 cells per cu. mm., 93 per cent of which were polymorphonuclear leukocytes; protein, 156 mg.; sugar, 82 mg. and chlorides, 628 mg. per 100 cc.; colloidal gold and Wassermann reactions negative; culture showed no growth. The patient died 9 days after the onset of neurologic signs.

Postmortem Findings

At autopsy there was bronchopneumonia of the bases of the lungs. Extensive lesions were found in the white and gray matter of the cerebral hemispheres. The blood vessels were surrounded by a cuff of inflammatory cells, about half of which were polymorphonuclear leukocytes. The inflammatory reaction was marked within the walls of the vessels or confined to the perivascular spaces, but occasionally the inflammatory reaction extended into the brain tissue (Fig. 1). About half of the cells in the exudate were polymorphonuclear leukocytes, whereas the remainder were macrophages with inclusions, lymphocytes, a few plasma cells, glial cells and gitter cells (Fig. 2). There was some variation in the type of inflammatory cells in different regions. Some vessels were surrounded only by mononuclear cells, apparently lymphocytes. In some places the majority of the cells surrounding vessels were gitter cells. In sections stained for myelin sheaths there were areas of perivascular demyelination (Fig. 3) similar to those found in equine encephalomyelitis and postinfectious encephalomyelitis (postmeasles or postvaccinal encephalitis). Observations in the literature and my own experience indicate that in postinfectious encephalitis extensive involvement of the brain in the nature of perivascular demyelination and infiltration is also accompanied by a similar involvement in the spinal cord. In this case sections of the spinal cord stained by myelin sheath and cellular stains were normal. There was no perivascular infiltration and no areas of demyelinization. In addition, there was only moderate involvement of the cerebellum, consisting chiefly of hyperemia. Only occasionally could polymorphonuclear leukocytes be found around some vessels and within the cerebellar tissue. These latter observations coincide with those of Wesselhoeft, Smith and Branch [4] and Farber, Hill, Connerley and Dingle [5] in cases of equine encephalitis in which

the spinal cord and cerebellum were not at all or only moderately involved.

Most of the lesions in the case here reported were within the white matter. But the gray matter also was involved. In scattered regions of the cerebral cortex capillaries were surrounded by polymorphonuclear leukocytes and in adjacent sections accumulations of glial cells and polymorphonuclear leukocytes could be observed around injured ganglion cells (Fig. 4).

Cultures of the nerve tissue and heart's blood were sterile. The spinal fluid, by culture and by intraperitoneal inoculation of guinea pigs, gave negative results.

Comment

This material presented a pathologic picture which could not be differentiated from that of equine encephalomyelitis in man. No attempt was made to isolate the virus. Therefore, one cannot be sure that this was a case of equine encephalomyelitis. The other possibility is that this case falls into the group of postinfectious encephalitides and the etiology may be related to the bronchopneumonia. But, as has been pointed out, the nature and distribution of the infiltrations is unlike that seen in encephalitis following such infections as measles, and the encephalitis accompanying or following pneumonia is usually a "toxic encephalitis" without any inflammatory reaction or myelin destruction.

It is not known why certain epidemic diseases lie dormant for many years and then suddenly attack the population. For this reason it seems to be important to trace sporadic cases, as this one might well be, with the hope of gaining some insight into the latency and outbreak of an epidemic.

Summary

A case is reported with a clinical, serologic and pathologic picture which could not be differentiated from that of equine encephalomyelitis in man. No attempt to isolate the virus of equine encephalomyelitis was made. Therefore, a definite differential diagnosis between sporadic equine encephalomyelitis and postinfectious encephalitis cannot be made, though the observations favor the former diagnosis.

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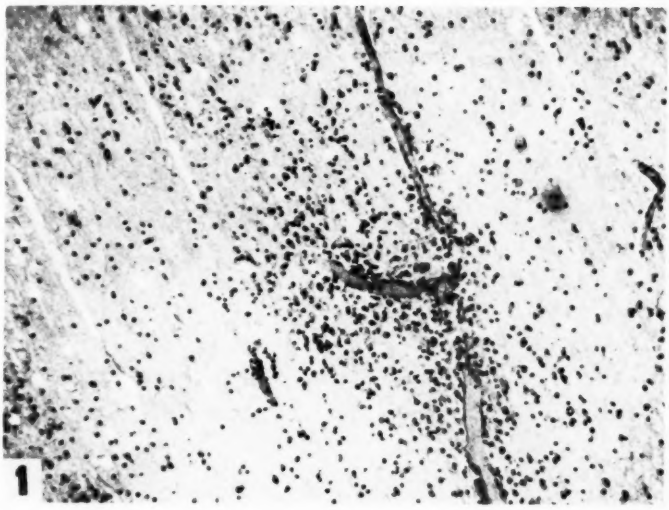
DESCRIPTION OF PLATES

PLATE 78

FIG. 1. Infiltrations around vessels and extending into the brain substance of the parietal lobe. Nissl stain. × 100.

FIG. 2. Perivascular infiltration around a vessel in the white matter of the parietal lobe. About half of the cells are polymorphonuclear leukocytes, whereas the remainder are macrophages with inclusions, gitter cells, lymphocytes, plasma cells and glial cells. Nissl stain. × 400.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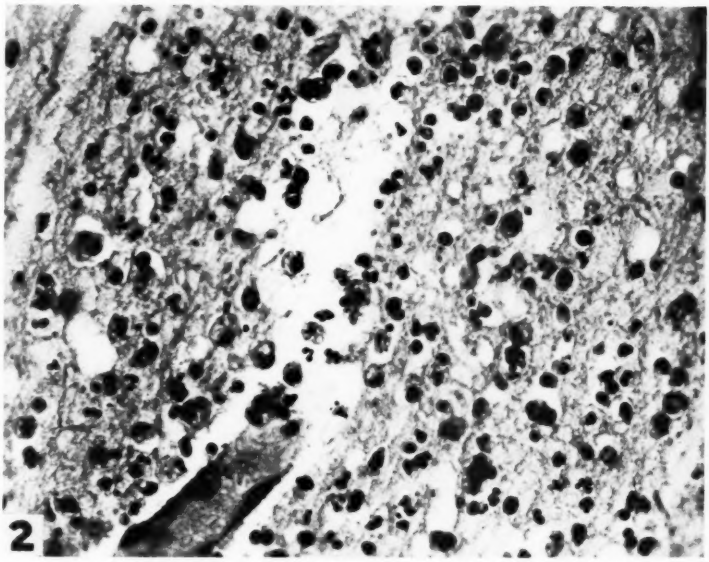
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PLATE 79

FIG. 3. White matter of the frontal lobe. Areas of perivascular demyelination, similar to those observed in postinfectious encephalitis. Weigert stain. × 5.

FIG. 4. Precentral cortex. Accumulation of polymorphonuclear leukocytes and glial cells around altered ganglion cells of the fourth and fifth cortical layer. Hematoxylin and eosin stain. × 400.

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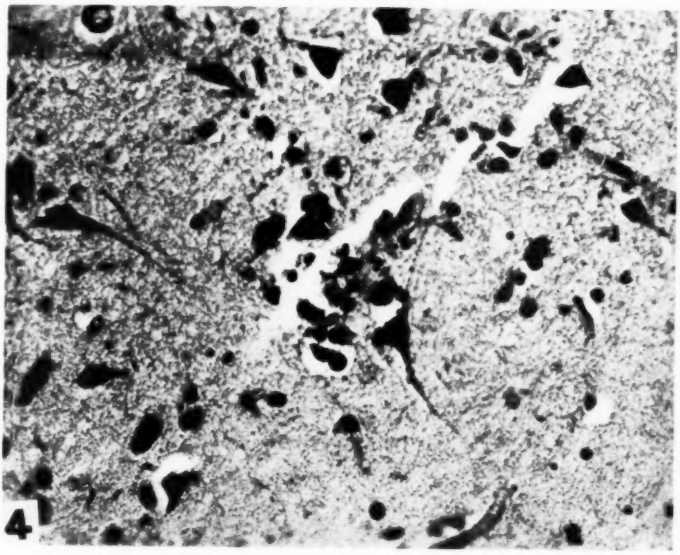

4

Adler

Equine Encephalomyelitis in Man

UNIV. OF MICH.

INFECTION OF NORMAL AND PASSIVELY IMMUNIZED CHICK EMBRYOS WITH CORYNEBACTERIUM DIPHTHERIAE*

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The use of the developing chick embryo as an experimental host for the study of bacterial infection was suggested by Goodpasture [1] in 1933. With Anderson [2] he demonstrated that *Corynebacterium diphtheriae* could be propagated on, and could induce a characteristic infection in, the chorio-allantois. Subsequent work by several investigators [3,4] in this laboratory has shown that for establishing some infections the embryo could be more directly approached by way of the amniotic fluid. The mouth and nasopharynx were in this way available for portals of entry. It appeared likely that *C. diphtheriae,* introduced into the amniotic fluid, would offer a new approach for the study of this infection with especial reference to the effect of passive immunization. This report will be concerned with observations made according to this plan.

EXPERIMENTAL PROCEDURE

The strain of *C. diphtheriae* used in these experiments was isolated at autopsy from a human case of diphtheria. Typical gross and microscopic changes of diphtherial infection with membrane formation in the pharynx, larynx, trachea and bronchi were present. Unprotected guinea pigs inoculated subcutaneously with 0.5 cc. of a 24-hour broth culture of this organism succumbed within 3 days. Other guinea pigs which had previously been passively immunized with diphtheria antitoxin survived following the injection of the same amount of culture.

The inoculum used in the chick embryo experiments consisted of 0.1 cc. of a 24-hour infusion broth culture of this organism. The cover-slip technic for preparing the chick embryos as described by Goodpasture and Buddingh [3] and the method of inoculating the amniotic fluid and body wall of the embryos as described by Polk, Buddingh and Goodpasture [4] were employed. Embryos of 15 to 16 days' incubation were used throughout these

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experiments. Forty-eight hours after inoculation, embryos were fixed *in toto* in Zenker's fixative (10 per cent acetic acid). The bacteria were demonstrated in sections after staining by Wright's method, as described by Goodpasture and Anderson.[2] Sections were also stained with hematoxylin and eosin for routine examination. Smears and cultures were made from the amniotic fluid 48 hours after inoculation.

For the purpose of studying the lesions in the nonimmunized host, 0.1 cc. of a 24-hour broth culture was injected into the amnion of 185 embryos of 16 days' incubation. At the end of 24 hours 124 were alive; 60 were alive at the end of 48 hours; none survived 72 hours. Twenty live embryos were fixed for microscopic study at the end of 48 hours. Smears and cultures from the amniotic fluid of these were positive for *C. diphtheriae*.

In order to study the effect of passive immunization on the infection, 64 embryos of 15 days' incubation were injected subcutaneously with 0.1 cc. of commercial diphtheria antitoxin. This amount equals about 250 units. Twenty-four hours later 0.1 cc. of a 24-hour broth culture was injected into the amniotic fluid of each of these embryos. Sixty-one survived 24 hours; 55 survived 48 hours; 14 were sacrificed at 48 hours for microscopic study; 30 survived 72 hours; 24 survived 96 hours; on the fifth day 16 embryos hatched and 8 were dead. As a control, 64 embryos of 16 days' incubation which received no antitoxin were given the same amount of culture by the same route. Forty-four of these survived 24 hours; 20 survived 48 hours, and 8 were sacrificed at 48 hours for microscopic study. The remaining 12 were dead at 72 hours. Smears and cultures from the amniotic fluid of the protected and unprotected embryos which were sacrificed at 48 hours were positive for *C. diphtheriae*.

To demonstrate serial passage of the infection, embryos of 14 to 16 days' incubation were used. Into the amnion of 8 embryos 0.1 cc. of a 24-hour broth culture was injected. Forty-eight hours later another group of 8 embryos was inoculated with 0.1 cc. of amniotic fluid from 2 live embryos of the first group. This procedure was repeated for five serial passages. Smears and cultures made at the end of 48 hours from the amniotic fluid of 2 or 3 live embryos of each group were positive for *C. diphtheriae*. No embryo used in this experiment survived 72 hours.

Pathological Observations

Gross examination of the embryos fixed for microscopic study showed no evidence of hemorrhage or necrosis in any of the organs.

Lesions in the Nonprotected Embryos

The microscopic sections from the 20 embryos fixed 48 hours after inoculation showed the following lesions:

Respiratory System. Clumps of bacilli were seen scattered throughout the nasal passages. A few necrotic or degenerating large monocytes and polymorphonuclear cells were found among the organisms. Some of the organisms were within the cytoplasm of these necrotic cells, but the majority were extracellular. The nasal mucosa adjacent to the bacilli showed lesions which varied from small areas of focal necrosis to large areas of ulceration. An inflammatory exudate consisting of large mononuclear cells, red blood cells and polymorphonuclear cells had replaced the destroyed epithelium. With a few exceptions the cells of this exudate appeared necrotic. The submucosa adjacent to the ulcerative lesions was infiltrated by monocytes and polymorphonuclear leukocytes. The blood vessels of the submucosa were markedly congested and areas of necrosis were seen. All of the embryos showed an inflammatory reaction of varying severity within the cranial sinuses. In some of them only focal areas of necrosis of the lining epithelium were noted. In others there was complete destruction of the epithelium, infiltration of the sinuses with an exudate similar to that seen in the nasal passages, and extension of the process into the bony wall of the sinuses with necrosis of bone tissue, which was infiltrated by an acute inflammatory exudate. A few extracellular bacteria were seen in the sinuses. The lesions found in the trachea of each embryo were similar to those of the nasal passages, consisting of masses of bacilli and a few necrotic phagocytes in the lumina, areas of ulceration of the epithelium and an infiltration of the submucosa by an acute inflammatory exudate. The primary bronchi showed the same type of change as seen in the trachea. An extensive pneumonia was noted in the lungs. The parabronchi and air capillaries were filled with an exudate of monocytes, polymorphonuclears and red blood cells. Most of the cells of this exudate appeared necrotic, and

small masses of bacilli were seen in the exudate. There was necrosis of many of the epithelial cells lining the parabronchi and air capillaries. Areas of necrosis and acute inflammation were seen also in the interstitial tissue of the lungs. There were areas of necrosis in the epithelium of the abdominal air sacs and a small amount of necrotic cellular exudate was present within them.

Digestive System. Small masses of bacilli were found in the oral cavities unassociated with any cellular exudate. The mucosa of the mouth and pharynx showed necrosis of a few cells but no areas of ulceration. The necrotic cells were more numerous in the basal layer of the stratified epithelium. In each of the embryos, lesions of the esophagus were seen which consisted of necrotic cellular exudate and small masses of bacilli in the lumen, areas of necrosis in the mucosa and congestion of the submucosa. Large masses of bacilli were found in the lumina of both the proventriculus and the gizzard. As in the other organs, a few phagocytes were seen among the masses of organisms and a small number of the bacilli appeared to be within the cytoplasm of these necrotic cells. There was superficial necrosis of the mucosa of the stomach and the large compound glands of the proventriculus showed focal areas of necrosis. No lesions were seen in the liver and intestines.

Circulatory System. Foci of necrosis were present in the myocardium. In these areas the nuclei were pyknotic, the homogeneous cytoplasm of the muscle fibers stained pink, and the stroma was infiltrated by monocytes and a few polymorphonuclears. No organisms were seen in these lesions. No lesions of the large blood vessels were found.

Bones. The only lesions of the bones were those which occurred adjacent to the involved cranial sinuses. These apparently represented an extension of the destructive process from the sinuses into the bone. No lesions were found in the sections of the long bones or the vertebrae.

The *spleen, adrenals* and *nervous system* showed only occasional necrotic cells. The necrotic nerve cells were found in the ganglia of the cranial nerves, none being noted in the central nervous system. The other organs showed no microscopic lesions.

Lesions in Passively Immunized Embryos

Respiratory System. In the nasal passages a few large mononuclear cells were found with an occasional rod-shaped bacterium in the cytoplasm of some of these cells. There was no necrosis of the nasal epithelium and no infiltration of the submucosa by an inflammatory exudate. The cranial sinuses contained a moderate amount of exudate, consisting chiefly of large mononuclear cells and a few polymorphonuclear leukocytes. The cells of this exudate showed no evidence of necrosis. In the cytoplasm of a few of the large monocytes were small clumps of bacilli. No extracellular bacteria were seen in the sinuses. The mucous membrane lining the sinuses was everywhere intact and the adjacent bony structures showed no necrosis. The tracheae contained a varying amount of exudate but in none of the sections was there any evidence of ulceration of the tracheal epithelium. In some of the embryos the entire lumen of the trachea was filled with an exudate consisting chiefly of large mononuclear cells. The cells were well preserved and many of them contained numerous bacilli in their cytoplasm. A similar reaction was seen in the bronchi. The lungs showed scattered areas of pneumonia. No necrosis of the parabronchial epithelium or any interstitial reaction was noted. The exudate in the parabronchi and air capillaries was of the same type as found in other portions of the respiratory tract. The cells were well preserved and a few of them contained bacilli. The abdominal air sacs contained a small amount of exudate but showed no necrosis or ulcerative lesions of the lining epithelium.

Digestive System. In the oral cavity and pharynx an occasional mass of bacilli was present. There was no cellular reaction and no evidence of injury to the mucosal lining of these passages. No ulcerative lesions were found in the esophagus of any embryo studied. Occasionally a small amount of cellular exudate composed chiefly of monocytes was present in the lumen.

Both the proventriculus and the gizzard of each embryo contained a large amount of cellular exudate and a few masses of bacilli in their lumina. This exudate also consisted chiefly of large monocytes and a few polymorphonuclear leukocytes. Many of the monocytes had phagocytosed small clumps of bacilli. Only

a few had undergone necrosis. There was no evidence of injury to the mucosa.

There were no areas of focal necrosis in the myocardium. No necrotic foci were seen in the spleens, adrenals, nervous systems, livers or bone marrow of the protected embryos.

Discussion

The scope of this report does not include a review of the numerous and extensive experimental studies performed with *C. diphtheriae* and its toxin in various experimental animals. A very complete review of this work was made by Andrewes, Bulloch, Douglas, Dreyer, Gardner, Fildes, Ledingham and Wolf [5] in 1923. These researches show that the mere application of cultures of *C. diphtheriae* to the intact skin or mucous membrane of experimental animals is rarely if ever capable of inducing experimental diphtheria. A previous injury to the surface is apparently essential and even under these circumstances it is not possible to carry on a series of passages, apparently because the microbes rapidly diminish in number after being introduced into the usual experimental animal. That this infection can be induced in serial passage from embryo to embryo was shown by Goodpasture and Anderson [2] who carried the infection for ten generations on the chorio-allantoic membrane. Serial passage of the infection can also be carried out when the embryos are infected by subamniotic inoculation.

Because of the sensitivity of the guinea pig to diphtheria toxin, our knowledge of the factors involved in the toxin-antitoxin reaction is quite extensive and adequate. We have, however, little experimental demonstration of the action of the microörganism at the sites of predilection where it is capable of producing the disease.

Following subamniotic inoculation, the susceptible chick embryo acquires the infection by means not very unlike those under natural circumstances in the usual human host, *i.e.*, by way of the mouth and nasopharynx. In this completely susceptible host the organism thrives in the fluids bathing the mucous membranes of the mouth, nasopharynx, trachea, lungs, esophagus and stomach. Sufficient toxin is apparently liberated to produce necrosis of the mucosa without a predisposing injury.

It is of importance to note that the essential characteristics of the disease as it occurs in the natural host, man, are reproduced in the developing embryo. Superficial growth of the bacillus without invasion takes place. The diffusion of toxin produces necrosis and this in turn enhances the growth-promoting character of the environment. Absorption of the toxin into the blood stream takes place and necrosis in the myocardium, adrenals, spleen and peripheral ganglia results from its selective pharmacological effect upon specific types of tissue.

It is unusual for the organism to produce a lesion in the stomach of the natural host, apparently because the gastric juice is inimical to the growth of the bacillus, but in this experimental host abundant growth occurs in the gastric fluids and lesions of the mucosa are produced. The development of the lesion does not depend primarily on the presence of a *locus minoris resistentiae* but on the availability of suitable environmental and growth factors.

These factors concerned in the pathogenesis of diphtherial infection are further emphasized in the passively protected embryo. It is significant in the first place that the antitoxin persists in the embryo as it does in other animals. The establishment of the disease in the unprotected host depends on the production of sufficient toxin. This, by its injurious effect, is responsible for the necrosis of the adjacent mucous membrane and the ensuing inflammatory reaction. The degenerating exudate apparently provides an excellent medium for the further rapid proliferation of the microörganism and increasing toxin production. In the passively immunized animals the neutralization of the toxin modifies the infection markedly. There is a less suitable environment for growth, and proliferation progresses at a reduced rate. The presence of the secretions of the intact mucous membranes, the absence of necrotic tissue and serum, and the phagocytosis of the organisms by large mononuclear cells which remain viable, are the important factors which result in the reduced rate of proliferation of the bacteria. The possibility that some antibacterial factor of the nature of opsonins or tropins may be supplied by the antitoxic serum must be considered, but such an assumption does not seem to be necessary to explain the alteration of the infectious process. The bacteria are not necessarily more sus-

ceptible to phagocytosis, but the phagocytes remain viable and are able to perform their function in the absence of toxin. Hammerschmidt,[6] who investigated the reaction to the growth of *C. diphtheriae* in a nodule of nutrient agar introduced subcutaneously in guinea pigs, observed that phagocytosis of the organisms by large monocytes in passively immunized animals is an important factor in reducing the rate of proliferation of the organisms. He was of the opinion that neither bactericidal substances nor opsonins played an important part in resistance to the infection under these circumstances.

In the chick embryo these well-recognized factors in the pathogenesis of diphtheria are subject to demonstration in a way until now not possible in any other experimental animal. Some of the factors concerned in the mode of action of diphtheria antitoxin are clearly emphasized. This host was used by Buddingh and Polk [7] to assay meningococcus antisera and also to analyze certain factors concerned in the protection conferred by these substances against infection by the meningococcus. Since a modified lesion may be produced in chick embryos by the use of therapeutic agents such as diphtheria antitoxin, the possibility of using this host for studying the mode of action of chemotherapeutic agents is suggested.

Summary

1. The developing chick embryo is susceptible to infection with *C. diphtheriae* when the mouth and nasopharynx serve as a portal of entry. The resulting lesions simulate those of the natural infection in man. Diphtherial infection of chick embryos can be carried on in serial passage when the infection is induced by subamniotic inoculation.

2. Some of the factors concerned with pathogenesis of diphtheria and with the protective mechanism produced by the administration of antitoxin are subject to analysis by a comparative study of the lesions found in unprotected and in passively immunized embryos.

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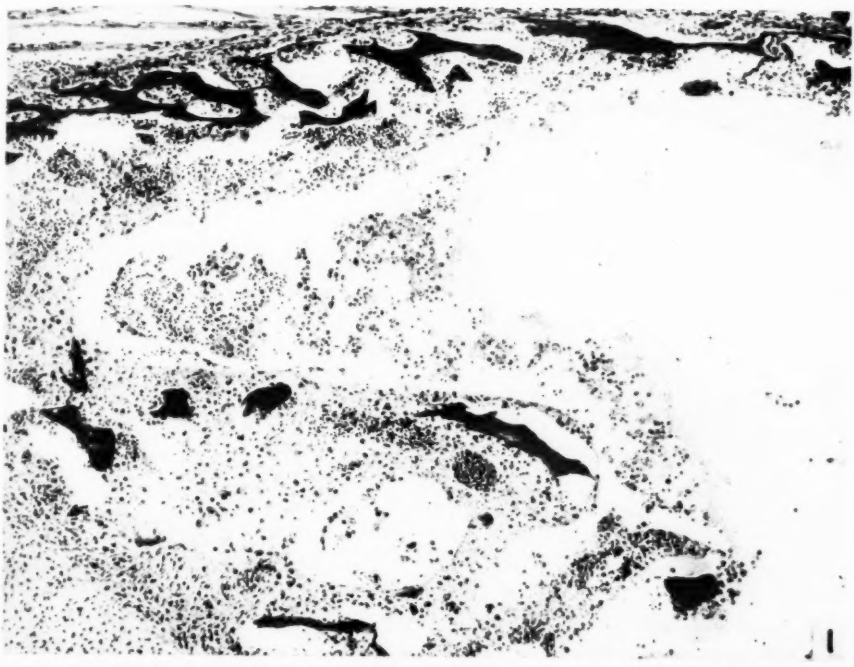
DESCRIPTION OF PLATES

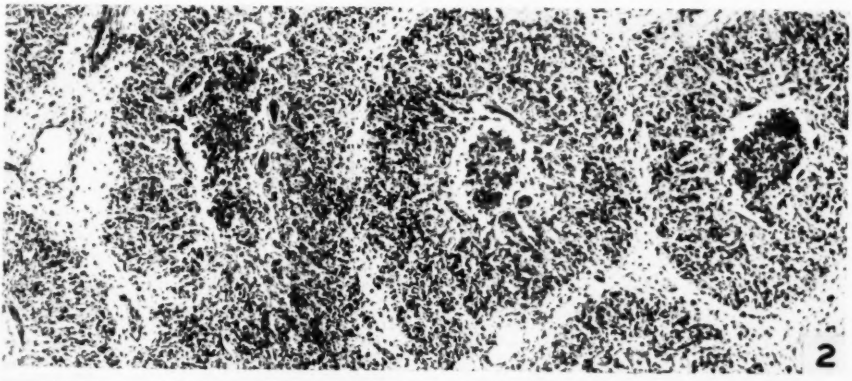
PLATE 80

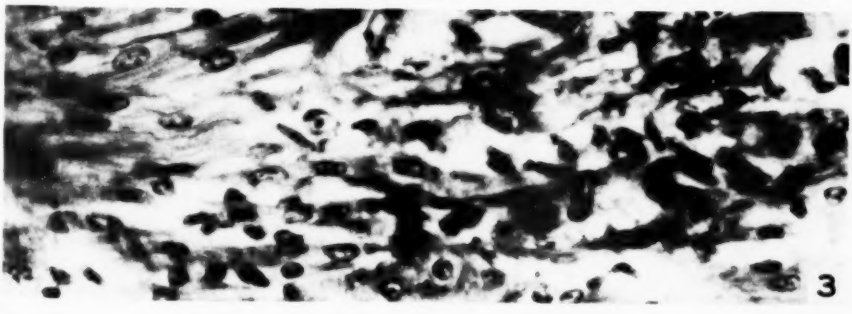
FIG. 1. Cranial sinus of nonimmunized chick embryo, infected with *Corynebacterium diphtheriae*, showing necrotic exudate and necrosis of mucous membrane and adjacent bone. Hematoxylin and eosin stain. × 120.

FIG. 2. Lung of nonimmunized chick embryo infected with *Corynebacterium diphtheriae* showing areas of necrosis in the lung tissue and necrotic exudate. Hematoxylin and eosin stain. × 120.

FIG. 3. Toxic necrosis in myocardium of nonimmunized chick embryo infected with *Corynebacterium diphtheriae*. Hematoxylin and eosin stain. × 550.







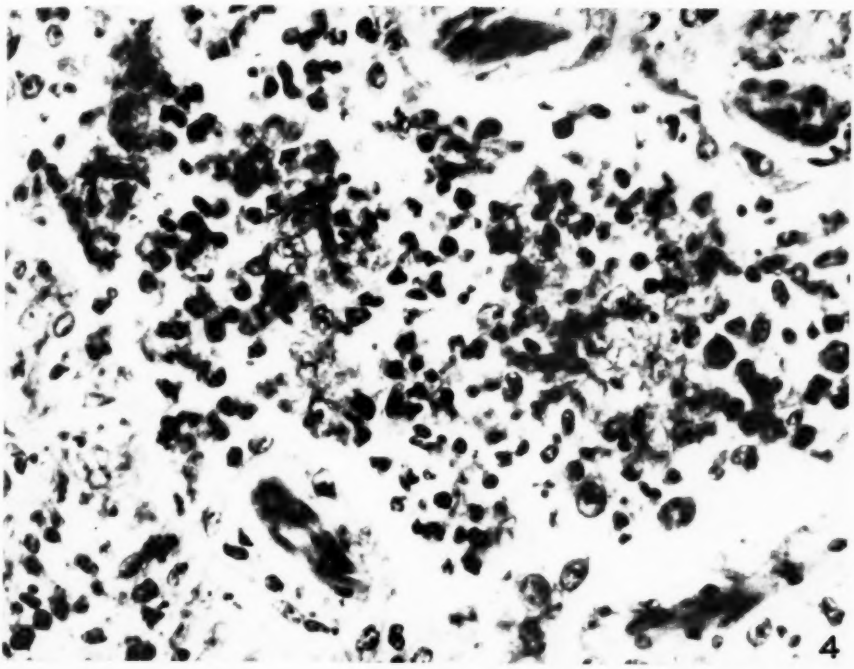
Cromartie

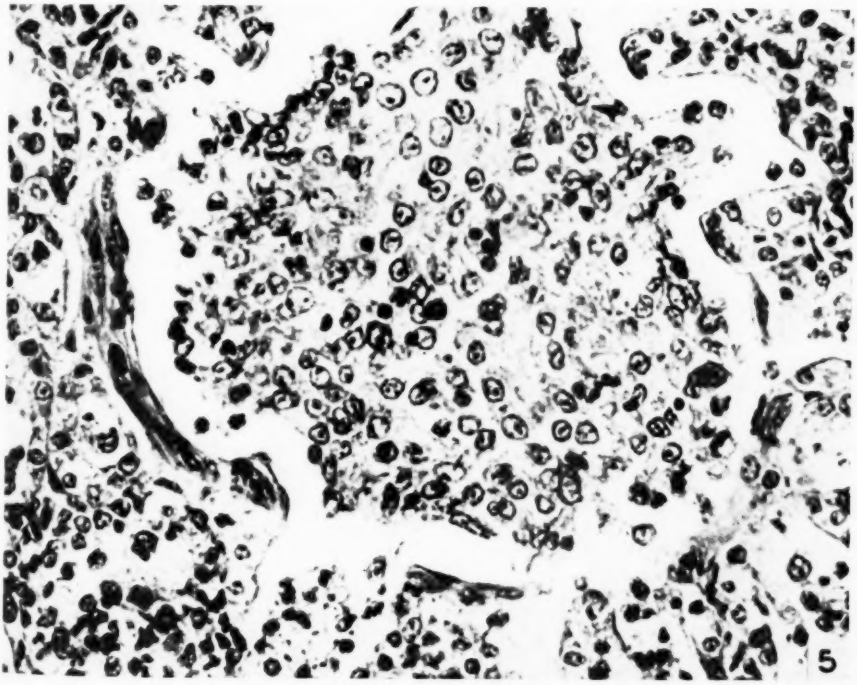
Infection of Embryos with *C. diphtheriae*

PLATE 81

FIG. 4. Lung of unprotected chick embryo showing necrotic exudate, necrosis of lung tissue, and masses of diphtheria bacilli. Hematoxylin and eosin stain. × 700.

FIG. 5. Lung of chick embryo, passively immunized with commercial diphtheria antitoxin, showing a monocytic exudate and minimal evidence of necrosis following infection with *Corynebacterium diphtheriae*. Hematoxylin and eosin stain. × 700.





Cromartie

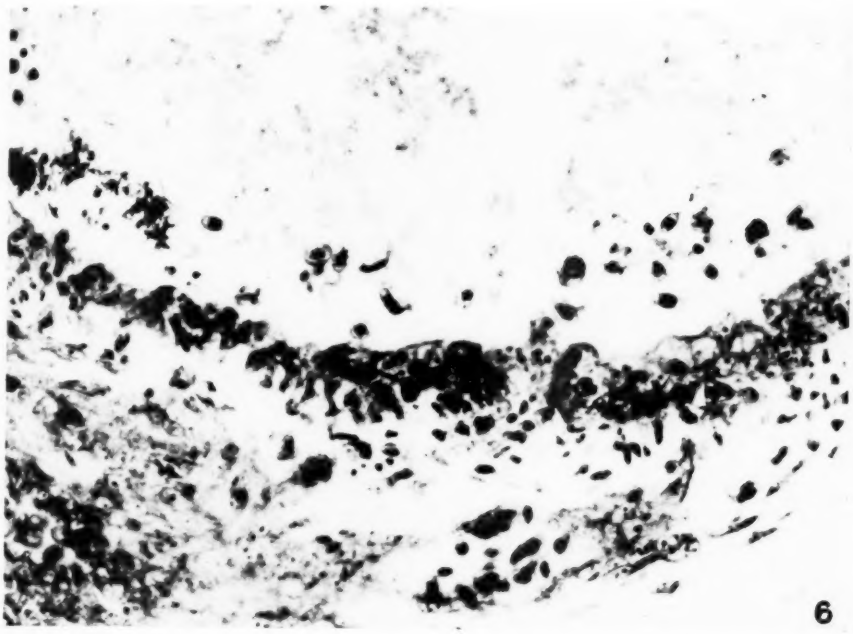
Infection of Embryos with *C. diphtheriae*

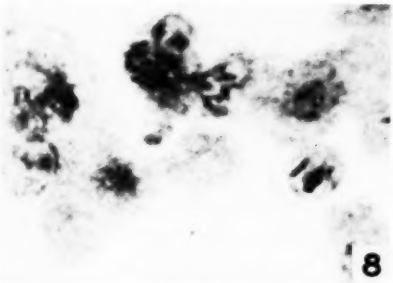
PLATE 82

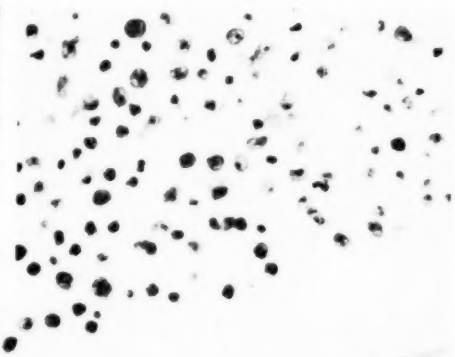
FIG. 6. Trachea of unprotected chick embryo showing masses of diphtheria bacilli, a minimal amount of necrotic exudate and necrosis of the mucosa and submucosa. Wright's stain. × 500.

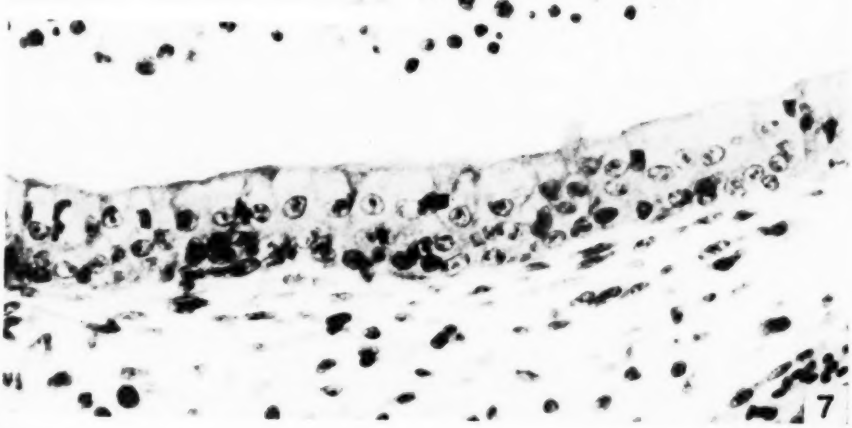
FIG. 7. Trachea of chick embryo passively immunized with commercial diphtheria antitoxin showing a monocytic exudate in lumen and minimal evidence of necrosis following infection with *Corynebacterium diphtheriae*. Wright's stain. × 500.

FIG. 8. Higher magnification of exudate shown in Figure 7 showing bacilli in the cytoplasm of large monocytes. Wright's stain. × 1900.









Cromartie

Infection of Embryos with *C. diphtheriae*

UNIV. OF MICH.

PHAGOCYTIC ACTIVITY OF THE OLIGODENDROGLIA AND AMPHICYTES IN THE BRAIN, SPINAL CORD, AND SEMILUNAR GANGLION OF THE MOUSE DURING INANITION*

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INTRODUCTION

The effects of starvation on the nervous system have been studied by various authors. The changes during rapid starvation, from complete deprivation of food, have been investigated in a number of species of experimental animals. Barrows [1] worked with rats; Urechia and Mihalescu,[2] with dogs; Garofeanu and Ornstein [3] studied the changes in rabbits, and de Couto e Silva [4] used frogs. Several reports on human material, however, have appeared. Among these are papers by Tarasevich,[5] Meyers [6] and Hassin.[7]

In two earlier papers [8,9] I have reviewed briefly the results of most of these investigators and have reported my own findings. All of the authors who have studied the effects of rapid starvation have described definite nerve cell changes such as loss of Nissl material, shrinkage, vacuolation and disappearance of cells. In my experimental work on the brains of mice,[8] using controls of the same age as the starved animals, I found, in addition to such changes, a marked grouping of the glial cells about nerve cells in the cerebral cortex, with an active process of neuronophagia or cytolysis of the nerve cells by these "satellite" cells.

In my first report on the subject,[8] I spoke of the cells active in the process of neuronophagia as being probably microglia, partly due to the classic conception of these cells. Further studies [10] and the employment of the silver technic have led me to the belief that the oligodendroglia are by far the preponderant element in this activity.

In the brain of a man who had wasted greatly due to obstinately refusing food over a period of 9 months I [9] found, in addition to the cytoplasmic changes of the nerve cells and the glial changes, a peculiar metamorphosis of the nucleus of the neuron. This consisted in a migration of the nucleolus to an eccentric position,

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followed by its disappearance, while at the same time the nucleus became very basophilic, resembling strongly the larger glial nuclei. In the present experimental study on mice I was interested in changes due to rapid starvation and also in those due to more prolonged starvation, in which this nuclear metamorphosis might occur. It seemed probable that a much shorter period of starvation might lead to such changes in mice, if they would occur at all. However, there was no way to verify this but by experiment.

In relation to the glial changes in starvation I wished to see whether the increase in satellitosis and the definite process of neuronophagia would occur in other parts of the nervous system, as in the gray matter of the spinal cord and in the semilunar ganglion. There was further interest in the possibility of definite evidences of neuronophagia in the ventral horn of the spinal cord and in the extent to which such changes might correspond to those reported in poliomyelitis. It was hoped to be able to determine whether the increase in glial cells is due to a migration from other regions or to an actual multiplication of cells in the region involved. Changes in the astrocytes were to be studied by silver carbonate impregnation.

Materials and Methods

In the study of the effects of rapid starvation, 8 mice were used. Four of these were pedigreed black male mice, of the strain C57, from the Jackson Memorial Laboratory. Four were white female mice. The black mice were each 110 days old when sacrificed, and the white mice were 20 days old.

For the study of the effects of more prolonged starvation, 6 white mice were used, 5 males and 1 female. These were litter mates, each 65 days old.

In the two groups of 4 animals used in the rapid starvation study, 1 mouse of each group was used as a control, being kept on an adequate and varied diet. The starved animals received all the water needed but no food whatever. Table I shows the degree and rate of weight loss in these animals. They were killed on the fifth day, the starved mice by this time having lost about 25 per cent of their original weight.

The mice used for the study of the effects of more prolonged starvation were weighed every other day and after weighing were

TABLE I

Rapid Starvation Experiment. Rate and Degree of Loss of Weight

Animals	Weights on succeeding days (grams)				
	1st day	2nd day	3rd day	4th day	5th day
(*Black mice*)					
$B_1♂$ (control)	26.7	27.7	27.4	26.8	26.7
$B_2♂$	31.1	30.3	28.4	27.2	25.5
$B_3♂$	29.9	27.7	24.9	25.2	21.9
$B_4♂$	26.5	26.8	23.9	22.6	20.6
(*White mice*)					
$W_1♀$ (control)	10.5	10.5	10.9	11.5	12.2
$W_2♀$	10.7	9.9	8.8	7.8	7.9
$W_3♀$	10.1	9.5	8.3	7.7	6.8
$W_4♀$	10.7	9.4	9.3	9.4	8.4

fed a small amount of a commercially prepared laboratory diet. The gradual starvation was continued for 28 days. On the 29th day they were given a final weighing and then sacrificed. Table II shows the average weight for each three successive weighings, made every other day, and the final weight on the 29th day. While there were rather large variations, the loss of weight in general was fairly constant and amounted to between 25 and 30 per cent of the original weight.

The animals were killed by rapid etherization. I had found in earlier work [11] that such etherization had no noticeable effect on the appearance of the nerve cells. Decapitation might have seemed preferable, but after such a process a clean removal of the spinal cord and semilunar ganglia is impossible.

TABLE II

Prolonged Starvation Experiment. Rate and Degree of Loss of Weight

Initial weight	Averages of 3 weighings (Column 1 shows average of 1st 3 weighings, column 2 of 2nd 3 weighings, etc.)					Final weight (Weight at time of killing)
(White mice)	1	2	3	4	5	
$WP_1♂$ (control) 20.0	19.8	19.7	20.3	19.9	22.4	25.8
$WP_2♂$ 22.0	21.1	17.2	15.2	14.5	13.4	13.4
$WP_3♂$ 19.5	16.1	16.1	16.6	17.6	15.4	14.2
$WP_4♂$ 19.5	18.4	14.8	14.6	14.6	13.8	13.4
$WP_5♀$ 20.0	15.8	15.3	14.9	13.7	14.4	14.9
$WP_6♂$ 22.5	20.4	17.1	15.3	15.1	14.7	17.7

Immediately after death the brain was removed, together with the cervical and a part of the thoracic portion of the spinal cord. The brain was separated from the cord and cut in half in the sagittal plane. The right half of the brain, together with the cord, was fixed in a 4 per cent aqueous solution of formaldehyde for Nissl staining. The semilunar ganglia on both sides were dissected out and placed in the same solution.

The procedure was the same for all animals except that the left halves of the brains were handled differently in the groups for the study of rapid and of prolonged starvation. Those in the former group were prepared by silver carbonate impregnation, to demonstrate astrocytes and oligodendroglia. Those in the latter group were prepared by Feulgen's method, specific for chromatin, as I was particularly interested in possible nuclear changes in this group. The method followed here was described by Ludford.[12]

The tissues of the 4 black mice used in the rapid starvation experiment were carried through the technical processes, up to embedding, in the same container, with the same fluids, separated by glass partitions with ample room below and at their margins for the free interchange of the reagents. The same procedure was followed for the white mice subjected to rapid starvation and the tissues of the animals suffering a more prolonged starvation, together with their control, likewise were carried through the steps in technic together.

After sectioning and spreading, the sections from the members of each of the three groups were carried through the process of Nissl staining in the same glass slide tray. The sections were stained for 75 minutes in a 1.5 per cent aqueous solution of cresyl violet.

The silver carbonate preparations of the left halves of the brains, designed to demonstrate astrocytes and oligodendroglia, were prepared according to the following method:

1. Fix in formaldehyde-ammonium bromide solution for 24 hours to several days.
2. Cut frozen sections of brain (sagittally, in this case) at 20 to 25 μ.
3. Place in 0.4 per cent aqueous solution of formaldehyde for temporary storage.
4. Wash for 1 minute in distilled water to which 1 drop of strong ammonia for each 5 cc. has been added, to neutralize the formaldehyde.

5. Wash in distilled water.
6. Place section in a 5 per cent sodium carbonate solution for 1 hour.
7. Wash in distilled water.
8. Impregnate in silver carbonate solution. This solution was made up as follows:
   a. Silver nitrate, 30 cc. of a 10 per cent solution.
   b. Sodium carbonate, 120 cc. of distilled water containing 10 gm. of sodium carbonate.
   c. Ammonium hydroxide sufficient to dissolve the precipitate.
   d. Distilled water to make the solution up to 450 cc.

   The impregnation was carried out *in the incubator* at a temperature of 38° C. for a period of 12 to 15 minutes. Heating during impregnation was a feature of the Spanish methods.[13]
9. Rinse in distilled water.
10. Reduce in a 2 per cent aqueous solution of formaldehyde until the sections are a yellowish gray.
11. Wash in distilled water.
12. Tone in gold-chloride (1:500).
13. Fix in 5 per cent solution of photographic sodium hyposulfite for 5 minutes.
14. Wash in distilled water.
15. Dehydrate on slide with 95 per cent alcohol followed by carbol-xylol-creosote mixture (1:15).
16. Mount in balsam.

All sections were examined both with lower powers and with the oil immersion lens.

Observations

Rapid Starvation

B_1, the control black animal, showed no evidence of degeneration in the cells of the brain, which resembled those of other normal mice of this age, with fairly abundant Nissl material, few satellites, and no neuronophagia. In B_2, B_3, and B_4, the starved animals, the nerve cells in practically all regions of the brain showed degenerative changes, consisting chiefly in chromatolysis and vacuolation. Even more conspicuous, however, was the change in the glial cells, particularly in the oligodendroglial cells. These appeared on low power observation to be increased in number and to have an increased tendency to phagocytosis, clustering about the nerve cells and invading their bodies, apparently by cytolytic action (Fig. 1).

The general picture was very similar to that described in my previous work. There was somewhat less neuronophagia in the

cerebral cortex of these starved animals than in those studied earlier, but it was very abundant in the deeper parts of the cerebrum and in the medulla oblongata. Here, as before, I found no action of the glial cells on the Purkinje cells of the cerebellum. These cells, however, did show chromatolysis and shrinkage in the starved animals.

Study of these brains has led me to the conclusion that the increase in numbers of the glial cells is due to amitotic division of these cells in the starved animals. In many instances two nuclei were seen lying in close juxtaposition as though they had just divided, and by more careful searching I found instances of division actually occurring. One of the more striking examples of such division is seen in Figure 2. This division is fairly typical although frequently the nuclei are considerably farther apart at the completion of division than in this picture.

It seems probable that the stage of most active division already was over in these animals, since the rapidly starved animals were already in the last stages of inanition when killed. In the brains of these starved animals I found amitotic division not only in the gray matter but also in the long chains of oligodendroglial cells between the nerve fibers in the white matter.

In the control white mouse (W_1, rapid starvation) the nerve cells were similar to those in other normal animals of 20 days, most of the cells having large amounts of Nissl material and many having fairly clear, white, vesicular nuclei. In the starved animals, W_2, W_3, and W_4, there were numerous degenerate-appearing nerve cells in the brain with many instances of neuronophagia, again more abundant in the deeper portions of the cerebrum and in the medulla than in the cortex. The increase in glial cells and in the tendency to satellitosis was not noticeably different than in the black mice 110 days old. As in the black animals, there was no neuronophagia of the Purkinje cells, but the loss of Nissl material in these cells in the very young starved animals was striking.

Studies of the glial cells (astrocytes and oligodendroglia) as seen in the silver stain, showed a surprising lack of change on the part of the astrocytes. In all of the starved animals these cells appeared similar to those of normal animals, with long cytoplasmic processes, many of them ending in sucker feet (Fig. 4).

There were no evidences of clasmatodendrosis (loss of the processes).

The oligodendroglia exhibited a great degree of polymorphism in both normal and starved animals, but in the starved mice they showed also the tendency to divide, to cluster in greater numbers about nerve cells and to hypertrophy, becoming rounder and apparently of a thinner consistency than normally, with cytoplasmic girders connecting the nuclei to the cell wall.

Ventral Horn of the Spinal Cord

Study of the spinal cords of these animals revealed marked differences between the control and starved mice, making the sections from each easily recognizable even with low power observation. In B_1 (control) the large motor cells of the ventral horn contained abundant Nissl material in the form of elongated blocks. The cell outlines were smooth. There were no signs of degeneration, satellitosis was not marked, and there was no neuronophagia. In B_2, B_3, and B_4 the majority of the large motor cells were hypochromatic and vacuolated. There were many satellite cells and conspicuous neuronophagia (Fig. 3). The picture was strikingly different from the normal.

Likewise in W_1 (control) the motor cells were in good shape and even more abundantly supplied with Nissl material than in B_1. There was little satellitosis and no neuronophagia. In W_2, W_3, and W_4 the motor cells were considerably altered. While many of them were hypochromatic, others appeared to have undergone a pyknotic shrinkage, with both nucleus and cytoplasm a deeply basophilic, almost homogeneous mass. The glial cells were increased, satellitosis conspicuous, and many instances of neuronophagia were seen.

Semilunar Ganglion

In the young, amply fed animals, both black and white, the nerve cells of the semilunar ganglia showed smooth outlines, abundant Nissl material in the form of granules or blocks of varying size, and, in the larger cells, frequently clear white, vesicular nuclei, which had conspicuous nucleoli.

The cytological picture of the semilunar ganglia of the starved animals was of a more varied type than in the spinal cord. In

B_2 the nerve cells appeared definitely altered, the chief difference being a diffuse, powdery appearance of the Nissl material. A few of the cells appeared very degenerate, with ragged cytoplasm, and in the neighborhood of such cells there was a definite increase in the amphicytes—interstitial cells with large, oval, vesicular nuclei. The nuclei of the nerve cells did not show any consistent differences between the starved and the fed animals.

In B_3 the Nissl material was definitely less abundant than in B_1 and B_2. There was in addition an irregular vacuolation in perhaps 20 to 30 per cent of the cells. This appeared to be a definitely degenerative change with the smaller vacuoles growing larger, or fusing to form larger vacuoles, until little or none of the normal cytoplasm was left. The amphicytes here were more definitely increased in number and more markedly aggregated about the degenerate cells than they were in B_2. Again, the nuclei did not show consistent differences, although in the more degenerate cells they never presented the clear white, vesicular appearance seen in numerous cells in the normal ganglion, nor were the nucleoli as conspicuously stained in degenerating cells. The Nissl substance was of the diffuse powdery type, apparently not greatly decreased in amount as compared to B_1. The coarse, irregular vacuolation seen in B_3 was as frequently present here. So too were the amphicytes about the degenerate cells. The nuclei were similar to those in B_3.

In W_2, W_3, and W_4, the starved white mice, the Nissl substance was greatly decreased in amount in the majority of the nerve cells. In many cells the large vacuoles of degeneration were present and the cytoplasm was of a ragged appearance, having pulled away at many points from the surrounding capsule. The amphicytes, greatly increased in numbers and gathered about the degenerating cells, could be seen to have invaded the capsules of such cells and apparently were phagocytizing them by a process of cytolysis. While it was impossible to determine in the majority of the cells whether the chromatolysis had been of the centripetal, centrifugal, or of an irregular variety, in a small proportion it was clear that the centripetal variety was occurring, while none of the centrifugal type could be seen. The nuclei of the cells in the semilunar ganglia of the white mice showed no consistent change. In the nondegenerate cells they seemed, if

anything, to be of a clearer white than in the normal animal, while in the more degenerate cells they appeared to share in the degeneration rather late, by alterations in the nucleolus and warping of the nuclear membrane.

PROLONGED STARVATION

The findings in the brains of these animals will be considered first. In WP_1 (control) the brain presented the normal appearance for an animal of this age (65 days). The cells of the cerebral cortex, the Purkinje cells, and the other cell groups contained abundant Nissl material. The Purkinje cells were of average size, but their nuclei were more uniformly basophilic than those of many young mice which I have examined.

In WP_2, WP_3, WP_4, WP_5, and WP_6 the cells of the cortex and of other areas in the cerebrum were definitely shrunken in appearance, and the nuclei more basophilic than in WP_1. In addition to the increased basophilic properties of the nuclei, in some of the cells (6 to 8 per cent), the nucleoli had become so eccentrically placed within the nuclei as to lie in contact with the nuclear membrane. There appeared to be a definite increase in the number of glial cells (oligodendroglia) and there were numerous instances of neuronophagia, often several in one high-power field. Many instances of two glial nuclei lying in close apposition, as though recently divided, were seen, and examples of amitotic division in progress were found. The Purkinje cells presented an appearance very different from that seen in WP_1. They appeared to be predominantly of two types. One type was that with a small, shrunken, very deeply basophilic cell body and a nucleus so deeply staining that its boundaries were almost indistinguishable. In the other type the cell was often enlarged, somewhat rounded, and always deficient in Nissl material. The entire line of Purkinje cells gave the appearance of having "sunken" back toward the granular layer.

The left halves of the brains of these animals, stained by Feulgen's method for chromatin, showed an increased chromatin content of the nuclei in the cerebral cortex and other parts of the cerebrum, with a less constant increase in the chromatin of the Purkinje cells of the cerebellum. Just how far this increase in chromatin content corresponds with the increased basophilic prop-

erties of many of the nuclei seen in the sections stained with cresyl violet, it is as yet difficult to say. The subject demands further investigation, and studies along this line are planned.

Ventral Horn of the Spinal Cord

The motor cells of the ventral horn of WP_1 (control) had smooth outlines, abundant Nissl material, and, in most cases, clear nuclei. No evidences of neuronophagia were seen.

In WP_2, WP_3, WP_4, WP_5, and WP_6 the nerve cells presented a definitely altered, although not uniform, appearance. Most of them fell into one of two classes. In the first and more numerous class were found hypochromatic, vacuolated cell bodies and only lightly basophilic nuclei. In the second group, containing about one third of the motor cells, both nucleus and cytoplasm stained very deeply and the processes could be seen extending for considerable distances. The glial nuclei were increased in numbers and there was much satellitosis and definite neuronophagia, which, however, was not so striking as in the more rapidly starved animals.

Semilunar Ganglion

In WP_1 (control) the ganglion cells had abundant Nissl material in discrete blocks or granules, with clear white, vesicular nuclei in the larger cells and lightly basophilic nuclei in the smaller.

In the ganglia of WP_2, WP_3, WP_4, WP_5, and WP_6, there were degenerative changes in the cytoplasm of the cells, consisting chiefly in the replacement of large portions of the normal cytoplasm by coarse vacuoles of varying size, some individual vacuoles occupying over a fifth of the area of the section of the cell. Hypochromatism was a feature of a considerable number of cells, but was not outstanding in an examination of a section of the ganglion as a whole. About many of the more degenerate cells there were aggregations of amphicytes, apparently phagocytizing the nerve cells.

An interesting nuclear change was seen in many of the cells in the animals which had undergone prolonged starvation. The nuclei in perhaps one fourth of the cells were considerably reduced in size, sometimes to less than one half of that which would

be considered normal for a cell of corresponding size, and had taken on a peculiar, glassy appearance, with the nuclear sap a light green in color. The cytoplasm immediately surrounding such a nucleus was usually entirely devoid of Nissl material, while the periphery of the cell was abundantly supplied. Just how much of this appearance was due to an actual centrifugal type of chromatolysis and how much to simple reduction in size of the nucleus, with failure of the Nissl material to move inward to fill in the space thus left, it is difficult to say.

Discussion

The most important conclusions from the present work are those in relation to the nature and extent of the phagocytic processes in the parts of the nervous system here studied.

As a result of rapid starvation, an active process of neuronophagia, carried out predominantly by the oligodendroglial cells, has been found in the cerebrum, medulla oblongata and spinal cord of the mouse. The term neuronophagia is used here to mean a cytolysis and ingestion of the cell bodies of neurons.

Spielmeyer,[14] while he cautioned against confusing "pseudoneuronophagia" (appearance of phagocytosis in which the satellite cells simply happen to be particularly numerous about a nerve cell or group of cells, or even lie in shallow concavities in such cells) with true neuronophagia, nevertheless recognized a process by which glial cells penetrate and take up the cytoplasm of degenerating ("fluidified") nerve cells. He stated: "Die Gliazellen haben vielfach die Tendenz, in den verflüssigten Zelleib einzudringen und sich an die Stelle der zugrunde gehenden Ganglienzelle zu setzen; wir haben es hier mit einer echten Neuronophagie zu tun." The illustrations of the process in his text show almost undoubted oligodendroglial cells in this rôle.

From observations on this process in great abundance and in all stages both in the brain and spinal cord, I not only can agree with Spielmeyer's statement but can assert that it is the glial cells which in most instances bring about the "fluidification," or lysis, of the nerve cell, probably ingesting it while this process is occurring. Many of the nerve cells observed in process of being phagocytized, both in the brain and in the ventral horn, have

had cell bodies fairly intact except at the point of attack by the glial cell or cells.

It is of great interest that Spielmeyer [14] described the phagocytosis of nerve cells in early poliomyelitis (a classic example of "neuronophagia") as carried out by polymorphonuclear cells from the blood vessels, but as of the same nature as the process which he described for the glial cells. He said of this condition: "Auch hier ist es nicht ein primärer Angriff der Zytophagen auf eine gesunde Zelle, sondern eine Einschmelzung der zerfallenden Ganglienzelle durch 'Nekrophagen'." Later lymphocytes and glial cells enter the picture in poliomyelitis. If this is the method of phagocytosis or neuronophagia of the motor cells by these acceptedly phagocytic polymorphonuclear leukocytes of the blood, there is full justification for applying the term "phagocytes" or "neuronophages" to the glial cells which carry on an almost identical process.

The question of what types of cells act as phagocytes in the central nervous system under various conditions is an old one. One of the earliest mentions of the possible or probable rôle of the oligodendroglia, as well as of the microglia in this relation, is found in the report of Robertson [15] on his successful platinum impregnation of the "mesoglia," the glial cells exclusive of the astrocytes. Dr. Clouston, speaking for Robertson in his absence, said of these cells: "Sometimes they have no processes, sometimes two processes . . . The mesoglia cells seem to have a phagocyte action in certain pathological conditions." Most of Robertson's illustrations are typical pictures of the oligodendroglial and some are typical of microglial cells, as seen with the later silver stains.

Penfield [16] denied that the oligodendroglial cells can assume a phagocytic rôle. He would confine such activity to the microglia, which would agree better with their supposedly mesodermal origin, than with the apparently exclusively ectodermal origin of the oligodendroglia. Del Rio-Hortega [17] was inclined to homologize these cells with the cells of the sheath of Schwann and not to assign any phagocytic rôle to them. Cone [18] stated that the oligodendroglia, at least in acute disease of the central nervous system, show only regressive changes, and that this fact supports Penfield's classification of the interstitial cells into neuroglia (astrocytes and oligodendroglia, supposedly of ectodermal origin

and never acting as phagocytes) and microglia (supposedly of mesodermal origin and frequently undergoing progressive changes to undertake a phagocytic rôle).

Ferraro and Davidoff [19] believed that they had demonstrated conclusively, by silver impregnation and fat-staining methods, that the oligodendroglia can act as phagocytes and that such cells, as well as the microglia, are transformed into "gitter-cells." They did not, however, bring out the relationship of these cells to nerve cells during this process. Cramer and Alpers [20] reported that they had shown a definite phagocytic activity on the part of the oligodendroglia in secondary degeneration in the white matter of the cord, the oligodendroglia passing through a metamorphosis involving acute swelling, among other changes, and playing the predominant rôle in the breakdown and digestion of the degenerate nerve fibers. They compared the phagocytic-digestive activity of the oligodendroglia to that of glandular cells, a comparison which could be followed in my own observations on the digestion of the nerve cell body by these cells. They found in this process also a hyperplasia, or increase in numbers, of the oligodendroglia. Such a hyperplasia has been shown in my material by increase in numbers and by the finding of amitotic division figures.

It is of significance that De Castro [21] regarded the amphicytes in ganglia as similar to the oligodendroglia. He said of the function of the amphicytes (satellite cells) under pathological conditions: "The digestion or disintegration of the cytoplasm is undoubtedly the work of the satellite cells which perhaps release histolytic ferments elaborated by their cytoplasm." My observations on the semilunar ganglion agree with such a conclusion. I found, also, that oftentimes in these young starved animals the amphicytic nuclei may be spheroid as well as oval, thus increasing their resemblance to oligodendroglia.

Rydberg [22] has questioned an exclusively mesodermal origin of the microglia. Studying fetal brains, both of human beings and lower animals, he found both microglia and oligodendroglia having an ectodermal origin. This finding is noted here as showing that the classification of glial cells according to origin can as yet scarcely be taken as a hard and fast one, nor as one on which to base considerations of the probable rôles of the various types. Some few investigators have suggested that even the astrocytes

may become transformed into phagocytic cells. Thus Scholz [23] stated that in some very chronic conditions the astrocytes perform a function of phagocytosis, probably aided by the oligodendroglia. I have not seen any evidences of such activity, nor indeed of any changes in morphology, in the astrocytes of my animals, either of those starved rapidly or of those starved over a longer period.

The most interesting changes in prolonged starvation, in addition to alterations in the cytoplasm of the nerve cells and the phagocytic activity of the oligodendroglia, are the nuclear changes occurring in many, though by no means all, of the nerve cells. These consist in increased basophilic properties of the nucleus, due probably in part or entirely to an actual increase in chromatin as shown by Feulgen's method. These nuclear changes are shown more or less markedly in the brain, cord, and semilunar ganglia.

Summary and Conclusions

The oligodendroglia increase in numbers and carry on a definite phagocytic activity in the cerebrum, medulla oblongata, and spinal cord of the mouse, both in rapid and in prolonged starvation.

In the semilunar ganglion the phagocytic rôle is played by the amphicytes, cells bearing a resemblance to the oligodendroglia.

I describe this type of process as one of neuronophagia, a cytolytic destruction and ingestion of nerve cells by other kinds of cells, whether by polymorphonuclear cells, as in early poliomyelitis, or by glial cells, as in my own material.

In prolonged starvation there are definite changes in the nuclei of some of the nerve cells, consisting chiefly in increased basophilic properties, probably correlated with the increase in actual chromatin content.

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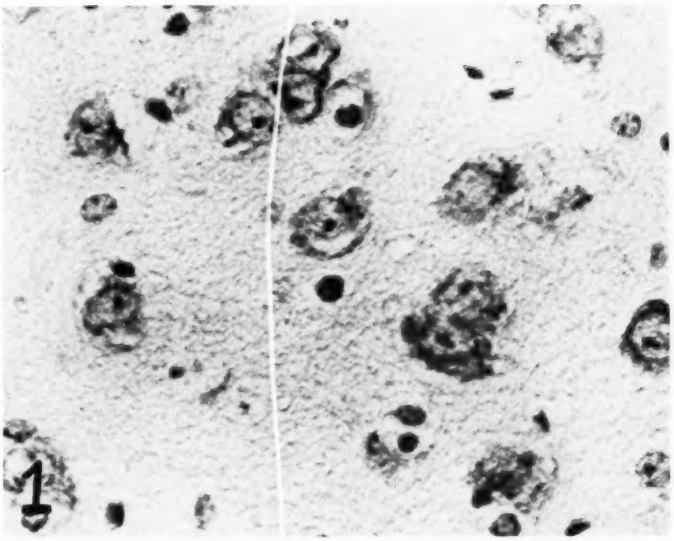
DESCRIPTION OF PLATES

PLATE 83

FIG. 1. Cerebrum of a mouse (B_2) starved for 4 days and in the last stages of inanition. The degenerating nerve cells are being invaded and phagocytized by oligodendroglial cells. Nissl stain. × 800.

FIG. 2. An oligodendroglial nucleus from the cerebral cortex of the same animal as in Figure 1, caught in the act of amitotic division. The daughter nuclei are not exactly similar here. Above the dividing glial nucleus is another oligodendroglial cell in contact with the crescent-shaped remains of an almost completely phagocytized nerve cell. Nissl stain. × 2000.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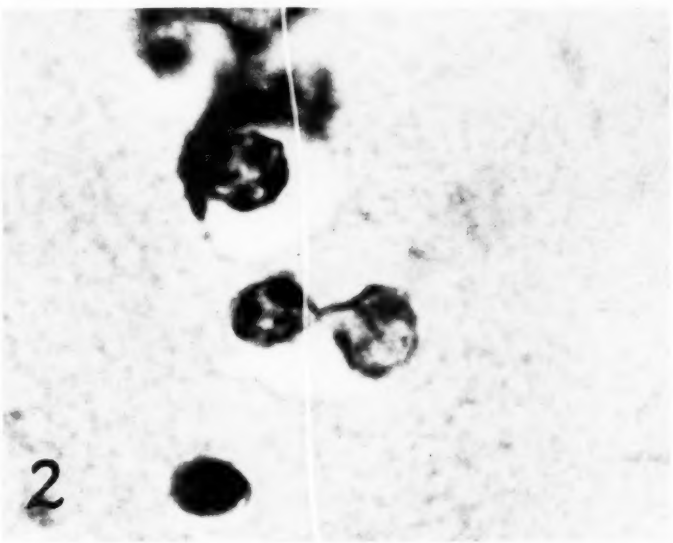
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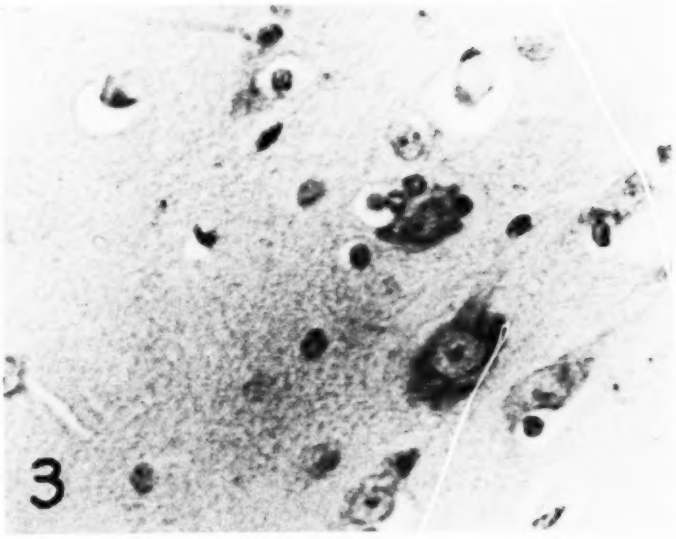
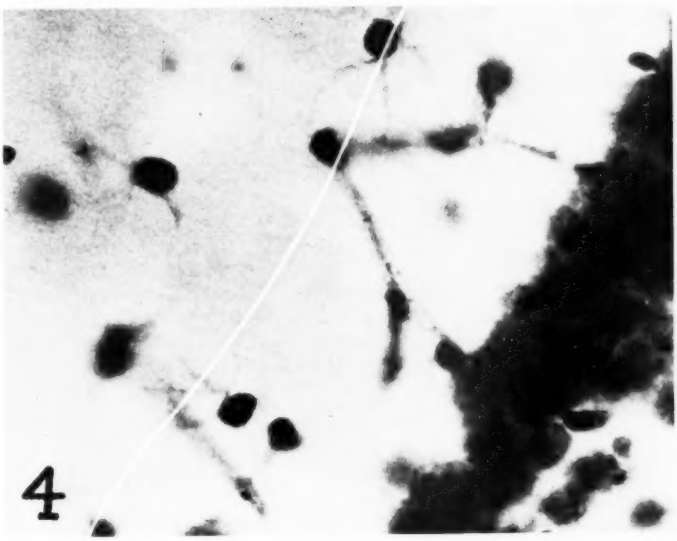
PLATE 84

FIG. 3. Ventral horn of the spinal cord of a mouse (B_2) starved for 4 days. The motor cells are degenerate and many of them are being phagocytized by the glial cells. Nissl stain. × 800.

FIG. 4. Astrocytes from the region of Ammon's horn of a mouse (B_3) starved for 4 days. There is no apparent change in these cells. Their processes and sucker feet appear as in normal animals. Silver carbonate method (see text). × 800.





UNIV. OF MICH.

AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

Twenty-Eighth Annual Meeting, Chicago, April 17–19, 1941

SHIELDS WARREN, *President*
H. P. SMITH, *Secretary*

ABSTRACTS OF PAPERS

EXPLANTATION AS AN AID TO THE STUDY OF KIDNEY PATHOLOGY. By Frederick M. Allen and William E. Youland. *From the Departments of Physiology and Pathology, New York Medical College, New York City.*

Explantation of kidneys under the skin was originally introduced by one of the writers with a view to broader purposes than have yet been carried out by authors who have subsequently adopted or claimed the method. This paper gives a preliminary description of some findings by this method, in particular, observations with temporary stasis of circulation and with diet.

THE INFLUENCE OF VARIOUS TYPES OF IMMUNIZATION UPON THE PATHOGENESIS OF EXPERIMENTAL ARTHRITIS. By D. Murray Angevine. *From the Department of Pathology, Cornell University Medical College, New York City.*

In the first part of the study, 107 rabbits were immunized, and 99 served as controls. The animals were immunized with either repeated intradermal or intravenous injections of heat-killed hemolytic streptococci over a period of 8 weeks. They were then infected with intravenous injections of homologous cultures. Histological studies were made on the joints, heart and kidneys in every instance. These experiments indicate that arthritis was produced more frequently and with smaller doses of bacteria in the intravenously immunized animals than in either the intradermally immunized or control rabbits.

The second part of the experiment, carried out on 81 rabbits, deals with the effect of intra-articular injection of heat-killed streptococci or nucleoprotein into one knee joint, and heat-killed staphylococci or horse serum into the contralateral joint. The animals were then infected intravenously with hemolytic streptococcus cultures and killed at various intervals. In agreement with the earlier work of Swift and Boots, no conspicuous differences were noticed in the clinical arthritis; however, cultural and histological studies were made on the synovial membranes of all the joints. It was found that the hemolytic streptococci were recovered with more difficulty from the intra-articularly injected joints, and although the clinical and gross findings were not conspicuous, the histological changes are of considerable interest.

TISSUE REACTIONS TO *Blastomyces dermatitidis* AND TO ITS PHOSPHATIDE AND POLYSACCHARIDE FRACTIONS IN ANIMALS. By Roger D. Baker. *From the Department of Pathology, Duke University Medical School, Durham, N.C.*

The tissue reaction in experimental blastomycosis of 2 to 6 weeks duration in mice differed from that noted in spontaneous human blastomycosis. In

mice the same cellular elements were involved, but minute polymorphonuclear abscesses and giant cells were not noted as in the natural human disease.

The phosphatide of the blastomycete produced in mice much the same tissue reaction as the living organism, but less necrosis, in the same periods of time. Thus the phosphatide appeared not to be responsible for the necrosis associated with the living organism.

The polysaccharide of the blastomycete was not studied to determine whether it had a necrotizing effect, but to see whether it produced the same acute effects in the rabbit as the tuberculo-polysaccharide, as described by Sabin, Joyner and Smithburn in 1938. It did. The blastomycete polysaccharide injected intraperitoneally into rabbits produced mild, sterile, transient, acute peritonitis; and in the blood, leukopenia, lymphopenia, and increase in immature polymorphonuclear cells. Control substances produced less pronounced changes.

FACTORS REGULATING THE ABSORPTION OF IRON IN DOGS AS MEASURED WITH THE RADIOACTIVE ISOTOPE. By W. M. Balfour. *From the University of Rochester, School of Medicine and Dentistry.*

It has been demonstrated that the anemic animal, deficient in iron stores, will absorb considerably larger amounts of iron than will the normal animal. The mechanism of this unusual physiological reaction has been studied from a number of angles. A typical set of experiments follows: A normal animal was shown to absorb 1.3% of a single dose of 130 mg. of iron containing the radioactive isotope. When this animal was rendered acutely anemic by the removal of two-thirds of the circulating blood volume during the course of a few hours, and twenty-four hours later given the same dose of radioactive iron, the amount of absorption was about the same as before, within experimental limits. After being allowed to return to a near normal range of circulating hemoglobin at the expense of the body stores of iron, and again fed radio-iron at the same dosage level, the amount of absorption was 10 per cent instead of less than 2 per cent. This suggests that the level of anemia, *per se*, did not influence the amount of iron absorbed, but that some tissue factor, possibly the mucosal iron of the intestine, was the determining factor.

EFFECT OF GLYCOGEN ON GROWTH OF WALKER 256 TUMOR IN RATS. By Howard A. Ball. *From the San Diego County General Hospital.*

Intravenous injection of commercial practical and chemically pure glycogen solutions cause marked inhibition of tumor growth, while starch, acacia, or glucose, in equal or higher concentrations, produce no effect. Hydrolysis of glycogen abolishes the effect.

NEURAL AND HUMORAL RESPONSES FROM HYPOTHALAMIC STIMULATION IN CAT AND MONKEY. By Morris B. Bender and Edwin A. Weinstein. *From the Laboratories of the Mount Sinai Hospital, New York.*

The hypothalamus was stimulated in cats and monkeys (*Macacca mulatta*) with the Horsley-Clarke technic. Observations were made on the normal pupil and completely denervated iris, and arterial tension. In the *cat* electric stimulation of the dorsal portions of the hypothalamus results in dilatation

of the normal pupil. This is due to parasympathetic inhibition since oculomotor nerve section abolishes this mydriasis. Stimulation of the ventral portions of the hypothalamus yields both neural and humoral "sympathetic" effects. The neural responses are: (a) prompt maximal dilatation of the normal pupil which is not abolished by section of the oculomotor nerve but is greatly reduced after interruption of the cervical sympathetic trunk, (b) retraction of the nictitating membrane, and (c) prompt increase in the blood pressure. None of these responses is abolished by adrenalectomy. The *humoral* responses are: (a) delayed mydriasis in the completely denervated iris occurring 9-20 seconds after the application and persisting after cessation of the stimulus, (b) delayed fall in blood pressure which is synchronous with the appearance of the mydriasis in the denervated iris. This is frequently preceded by the prompt (neural) rise in blood pressure. Bilateral adrenalectomy abolishes the delayed mydriasis and depressor effects. Intravenous injection of adrenalin, 0.001 gamma per kilo per second, reproduces the "humoral" effect obtained with hypothalamic stimulation.

In the *monkey*, the neural mechanism of pupillary dilatation is predominately that of sympathetic excitation. Stimulation in most hypothalamic areas produces a prompt mydriasis which is greatly reduced or abolished by section of the cervical sympathetic trunk. Parasympathetic inhibition is difficult to detect. The arterial tension is usually raised but never as high as in the cat. In marked contrast to the cat, conspicuous humoral effects are never seen; infrequently a slight delayed "humoral" mydriasis may be observed only after an intramuscular injection of cocaine.

THE EFFECT OF ATROPINE AND PILOCARPINE UPON THE SPHINCTER OF ODDI IN HUMAN SUBJECTS. By George S. Bergh. *From the Department of Surgery, University of Minnesota, Minneapolis.*

The effect of atropine and pilocarpine upon the sphincter of Oddi was studied in patients who previously had undergone cholecystectomy and choledochostomy. The resistance to flow of fluid from the bile duct into the duodenum was measured as "sphincter resistance."

In eleven experiments atropine sulphate was administered subcutaneously in dose of one-fiftieth to one one-hundredth of a grain. In ten cases there was no significant change in the intramural resistance, and in one case the resistance was slightly decreased.

In eight experiments the effect of the subcutaneous injection of six milligrams of pilocarpine was studied. In seven patients there was no change in the sphincter resistance, and in one case relaxation occurred.

These findings suggest that the parasympathetic nervous system does not play an important rôle in the regulation of sphincter activity in human subjects.

BLOOD PHOSPHOLIPID CHANGES IN EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS. By Jesse L. Bollman and Eunice V. Flock. *From the Division of Experimental Medicine, The Mayo Foundation, Rochester, Minnesota.*

Rabbits fed cholesterol for several weeks develop a tenfold increase in the cholesterol of the blood. The total phospholipid content of the blood increases to twice its normal value. Most of this increase is due to increases in

the choline-containing phospholipids (lecithin and sphingomyelin) and a slight increase in the cephalin of the blood. The phospholipid and cholesterol of the blood of rabbits receiving phospholipid in addition to the cholesterol added to the diet was similar to those receiving cholesterol alone. In rabbits which received phospholipid without added cholesterol, the blood phospholipid was elevated about 25% above the control values.

The rabbits receiving cholesterol developed marked atherosclerosis in from twenty to forty weeks. Microscopic lesions, similar to the early atherosclerotic changes, appeared in the aortas of rabbits fed phospholipids for 30 to 40 weeks although the blood cholesterol of these animals was not greatly elevated at any time during their course. These studies confirm the well established facts concerning cholesterol arteriosclerosis, and further suggest that the elevation of the phospholipids of the blood is a factor which merits further consideration.

DIFFUSION OF SOME ANIMAL VIRUSES. By Jaques Bourdillon. *From the Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania, Philadelphia.*

The author's analytical diffusion method has been applied to the study of viruses of influenza A, vaccinia, and mouse encephalomyelitis.

Diffusion of influenza (in mouse lung suspension). The results showed that the diffusing virus was not homogeneous, but consisted of two fairly distinct fractions: one fraction, representing about 99% of the total virus, diffused slowly and appeared to consist of particles with a diameter of the order of 200 millimicrons; the other fraction, representing about 1% of the total virus, diffused fast, and appeared to have a particle diameter of the order of 6 millimicrons.

Diffusion of vaccinia (from rabbit skin). The results were similar to those obtained with influenza. They suggested that most of the infectivity was present in particles of the order of 200 millimicrons in diameter, but that about 0.1 or 0.01% of the virus was present in particles about 6 millimicrons in diameter.

Diffusion of mouse encephalomyelitis (from mouse brains). Diffusion showed the material to be very inhomogeneous. About 10% of the virus present appeared to have a particle diameter of about 15 millimicrons; smaller particles may have been present.

The tentative conclusion has been drawn that the elementary bodies or particles currently described as the physical units of animal viruses may be only virus carriers, and that the actual virus units are of the size of low molecular weight proteins.

FURTHER OBSERVATIONS ON A GASTRIC SECRETORY DEPRESSANT FACTOR IN GASTRIC JUICE. By Alexander Brunschwig and Richard A. Rasmussen. *From the Department of Surgery, University of Chicago.*

Previous studies showed that achlorhydric gastric juices of patients with a gastric cancer, and of patients with pernicious anemia when injected intravenously into dogs with large gastric pouches produced a transitory reduction in secretion volume, and an achlorhydria in over 70 per cent of samples tested. The dose employed was 1 cc. of juice per kilo weight of the dog.

As controls, acid gastric juices, neutralized before injection, obtained from patients not exhibiting the above diseases, were employed and gastric secretory inhibition was observed in approximately 20 per cent of the samples.

The secretory depressant factor is present in the white flocculent precipitate obtained when four volumes of absolute alcohol are added to the juice. The incidence of samples among the controls exhibiting the gastric secretory depressant was thought to be too great to be regarded as "false positives." In order to test the hypothesis that the depressant found in the controls might be evidence of a normal chalone mechanism, hyperactive in carcinoma of the stomach and achlorhydria, and in pernicious anemia, precipitates from larger quantities of control samples of juices were injected into dogs, the results of which will be reported.

THE FUNCTIONAL ACTIVITY OF SMOOTH MUSCLE TUMORS OF THE UTERUS. By Charles S. Bryan and Shields Warren. *From the Laboratory of Pathology, New England Deaconess Hospital, Boston.*

No information has been available as to whether or not the smooth muscle cells of a leiomyoma can contract. The following study was carried out on strips taken from leiomyomas of the uterus and from the normal smooth muscle of the wall of the same uterus as controls.

The strips were excised promptly after operative removal of the specimen and immersed in oxygenated Ringer's solution kept at 38° centigrade, and the muscle strips attached by threads to a pointer on the kymograph. The lower ends of the strips were anchored. Contractions were obtained with pituitrin (¼ cc. in 250 cc. of Ringer's solution) from 7 leiomyomas, and with histamine (0.5 cc. of 1/1000 in 250 cc. of Ringer's solution) from 6 leiomyomas. Some strips failed to contract, and showed either much fibrosis, or muscle cells not in the long axis of the strip.

OBSERVATIONS ON THE INFECTION OF CHICK EMBRYOS WITH *B. tularense*, *Brucella* AND *P. pestis*. By G. John Buddingh and Frank C. Womack. *From the Department of Pathology, Vanderbilt University School of Medicine, Nashville.*

Developing chick embryos are susceptible to infection with *B. tularense*, *Brucella abortus*, *Brucella melitensis* and *Pasteurella pestis* by chorio-allantoic inoculation. Each of these infections induces characteristic reactions in the chorio-allantois and the embryo proper. Notable differences appear in the early stages of infection, especially in relation to the behavior of these micro-organisms in respect to various intracellular environments. In the infected chorio-allantois *B. tularense* exhibits a marked predilection for growth within the epithelial cells of the ectoderm. The *Brucella* strains show a much greater affinity for endothelial cells than for ectodermal epithelium. *Pasteurella pestis* grows readily within ectodermal epithelium and in the intercellular spaces. Generalization of these infections to the embryo proper induce characteristic and distinct lesions. Important features in the behavior of these micro-organisms in relation to the early stages of the pathogenesis of infection are emphasized by these observations.

STUDIES ON INSULIN-ALLERGY. By Paul R. Cannon and Charles E. Marshall. *From the Department of Pathology, University of Chicago.*

Experiments are described concerning the antigenic properties of insulin. Evidence is presented that, in patients proved by skin-tests to be allergic to insulin, the serum contains a specific antibody, demonstrable by the collodion particle agglutination method and by the Prausnitz-Küstner reaction. Discussion is directed to the possible relationship of this antibody to some instances of insulin-resistance.

A STUDY OF THE RELATIONSHIP BETWEEN THE VIRUS OF INFLUENZA A AND FILTERABLE COMPONENTS OF NORMAL LUNGS. By Leslie A. Chambers and Werner Henle. *From the Childrens Hospital of Philadelphia, and the Johnson Foundation, University of Pennsylvania, Philadelphia.*

Particles sedimented from Berkefeld filtrates of emulsified influenzal mouse lung by moderately high speed centrifugation possess the major part of all the infectivity of the original filtrate even after repeated washing. Normal mouse lungs yield quantitatively similar sediments. The normal and infectious lung sediments have been studied simultaneously by detailed chemical analysis, dark field and electron microscopy, specific staining, pycnometry, and spectroscopy. No significant differences between the normal and pathogenic particles have been found, excepting the infectious property.

Such evidence indicates that a component of normal lungs either acquires infectivity by some unknown structural rearrangement, or that it adsorbs an infectious unit of much smaller size than that indicated by ultrafiltration studies. Extremely high infectious titres in a small percentage of the purified preparations, and failure to sediment completely the infectivity of some mouse lung filtrates point toward the latter view. Furthermore, antiserum against particles derived from normal mouse lungs agglutinates extensively the infected preparations and the major part of infectivity is to be found in the agglutinate. This suggests a carrier function of the normal cell constituent.

As the result of a search for a source of virus relatively free from cellular elements, it was found that the extra-embryonic fluid harvested from infected chick embryos is usually fatal for mice in high dilutions. A large part of the infectivity fails to sediment under conditions which remove it almost completely from mouse lung filtrates. Sedimentation, and diffusion data indicate an infectious unit of relatively small size, probably of the order of 10 millimicrons in diameter.

Adsorption with normal lung particles removes most of the infectivity from the extra-embryonic fluid, and transfers it to the particles. This strongly supports the concept of a carrier rôle for the normal cell particles.

RELATION OF AGE TO THE SUSCEPTIBILITY OF THE ERYTHROCYTE TO HYPOTONIC SALINE SOLUTION. By W. O. Cruz. *From the Department of Pathology, University of Rochester School of Medicine and Dentistry.*

Radioactive iron when administered to an animal in which there is a stimulus for hemoglobin formation is promptly converted to new hemoglobin and remains in the red cell until that cell undergoes disintegration.

If the isotope is given in a single dose, the age of the red cells incorporating the radioactive hemoglobin can be determined. The physical and chemical properties of these labelled cells can then be compared with the same properties of the older red cells in the circulation.

It can be shown that the *newly-formed red cells* containing the radio-iron are much more susceptible to treatment with hypotonic saline than the other circulating cells of the dog. This susceptibility of red cells to hypotonic saline solution decreases progressively until at about three days of age the circulating red cells are all equally sensitive to hypotonic solution.

BACTERIOGENIC HEMAGGLUTINATION. By I. Davidsohn and B. Toharsky. *From the Department of Pathology, Mount Sinai Hospital, Chicago.*

It was previously reported that a gram-positive bacterium designated as *Corynebacterium H* was isolated, which produced panagglutinating properties in serum and plasma and panagglutinability of red cells. Various features of the newly developed antibody-like bacteriogenic property of the serum were described.

The present paper shows:

1. The pH of inoculated serums was found slightly lower than the pH of the uninoculated controls, but the change was not related to the development of bacteriogenic hemagglutinins.
2. Concentrations of certain bacteriostatic agents were established capable of inhibiting bacteriogenic transformation.
3. Yeast extract and certain components of the vitamin B complex favored the development of hemagglutinating properties in serums.
4. The component of the serum which is essential for the transformation by the bacterium was destroyed by a temperature of 65 C. The fraction of the serum which is needed for the development of the bacteriogenic agglutinins is used up in the process of transformation. After removal of the agglutinins by proper adsorption no further transformation occurred.

THE PHYSIOLOGICAL AND PHARMACOLOGICAL BEHAVIOR OF THE HUMAN APPENDIX. By Clarence Dennis. *From the Department of Surgery, University of Minnesota, Minneapolis.*

Previous work from this laboratory has indicated that experimental obstruction to the lumen is capable of producing acute appendicitis in man, the chimpanzee and the rabbit.

The muscular activity of the human appendix has been studied both in freshly excised specimens and in appendixes made available by appendicostomy.

Although the muscular activity of the appendix is somewhat less than that of the ileum, it was demonstrable in 29 out of 32 uninflamed excised specimens, less often in inflamed ones. The effects of a variety of drugs were found in general similar to those in the ileum.

Sufficient muscular activity is demonstrable to suggest that this may play a rôle in initiating appendicitis through luminal obstruction.

INFLUENCE OF DIET ON LIVER LESIONS CAUSED BY EXCESS DIETARY CYSTINE. By David P. Earle, Jr. and Joseph Victor. *From the Research Service, First Division, Welfare Hospital, Department of Hospitals, City of New York, and the Department of Medicine, College of Physicians and Surgeons, Columbia University.*

Albino rats fed a low fat (5 per cent) diet with 10 per cent cystine showed portal necrosis and hemorrhage after 2-3 days and portal cirrhosis of the liver as early as 5 days. Fatty infiltration of the liver occurred in rats surviving 6 or more days. These lesions were uninfluenced when this low fat diet was low in protein (5 per cent), high in protein (40 per cent) or high in yeast (20 per cent). High fat (25 per cent), low protein (5 per cent) with 10 per cent cystine produced longer survival and delayed the appearance of cirrhosis to 2 weeks but did not affect necrosis and hemorrhage. Fatty infiltration was greater than that of the preceding groups. Rats fed the McCollum stock diet with 10 per cent cystine showed less fatty infiltration and the incidence and severity of the acute and chronic lesions were diminished.

High fat (25 per cent), low protein (5 per cent) diet with 5 per cent cystine resulted in liver lesions resembling those following 10 per cent cystine but their incidence and severity was diminished. However, fatty infiltration was more severe. One per cent choline added to this diet depressed the fatty infiltration but increased the incidence and severity of the cirrhosis. The McCollum diet with 5 per cent cystine had the same effect as the 1 per cent choline in the aforementioned diet.

THE NON-NEOPLASTIC HEPATIC CHANGES IN RATS FED DIMETHYLAMINOAZOBENZENE WITH SPECIAL REFERENCE TO PIGMENT DEPOSITS. By Jesse E. Edwards and Julius White. *From the National Cancer Institute, United States Public Health Service, Bethesda, Maryland.*

More than 200 rats were fed dimethylaminoazobenzene (butter yellow) continuously and autopsied at varying intervals from the start of the experiment. Among the changes in the liver exclusive of tumor formation there is cirrhosis with the presence of at least two pigments, one containing iron and one free of iron. The latter pigment is insoluble in the usual organic solvents and is apparently of lipoidal nature.

The cirrhosis is associated with varying degrees of proliferation of bile ducts to the point of grossly visible areas containing no parenchymal cells and only numerous tortuous biliary channels. Cysts of varying size and number are present. These appear to be dilated bile ducts.

The demonstration included lantern slides showing the tinctorial reactions of the pigments and the appearance of the bile ducts and cysts.

EFFECTS OF HYPOPROTEINEMIA ON BLOOD PRESSURE IN NORMAL AND HYPERTENSIVE DOGS. By Cyrus C. Erickson. *From the Department of Pathology, Duke University Medical School, Durham, N.C.*

The following observations show that depletion of circulating plasma proteins in hypertensive dogs is associated with a depression of established blood pressure levels, and to a lesser degree a response of like nature is noted in normal dogs.

Normal and hypertensive dogs have been studied during periods of hypo-

proteinemia with daily observations of blood pressure levels. Hypertension was produced experimentally by the Goldblatt renal artery constriction method. Hypoproteinemia was induced by intravenous injections of 6 per cent gum acacia in Locke's solution. The plasmapheresis method was also used as a control method to obviate the known reduction of red cell volume, lowering of cholesterol, and liver injury which occurs with acacia administration.

Associated with hypoproteinemia a definite depression of blood pressure levels occurred in both hypertensive and normal dogs; the more conspicuous drop is demonstrated in the hypertensive dog. The total plasma protein level, the jugular hematocrit, and the fibrinogen show the characteristic drop in the acacia experiments described by several investigators. Plasma depletion experiments tend to indicate that hematocrit variations are not of significance in the depressor effect of hypoproteinemia associated with the acacia injections.

These observations suggest that the acacia administration has no specific pressor effect on the blood pressure, but that plasma protein depletion is probably the significant factor in the recorded blood pressure variations. It is our belief that we have quantitatively reduced specific blood protein or protein-like substances which are necessary components of the humoral mechanism maintaining a "pressor-depressor balance." These observations are compatible with recent experimental evidence of "chemical mediators" removed from the blood of hypertensive dogs.

THE RELATION OF THE PANCREAS TO THE CELIAC SYNDROME. By Sidney Farber and Charlotte Maddock. *From the Department of Pathology, Harvard Medical School, Boston.*

In the pancreases of more than 40 patients suffering from one variant of the celiac syndrome, called for convenience "pancreatic fibrosis," there has been found with regularity a series of changes beginning with an alteration in the character of the secretions in the acini (Wolbach) and passing through stages which include dilatation of ducts, atrophy of acinar structures and eventually marked fibrosis of the gland. Important interference with the formation and liberation of pancreatic enzymes was suggested by these findings. Accordingly, approximately 50 determinations have been made, in a preliminary survey, of the pancreatic enzymes (trypsin, amylase, lipase) in the duodenal contents of three types of patients: (1) normal infants and children, (2) patients suffering from the pancreatic fibrosis variant of the celiac syndrome, and (3) patients suffering from celiac disease. Pancreatic enzymes were greatly reduced or absent in the duodenal contents of four patients in whose pancreases histologic changes were found at autopsy (pancreas fibrosis group). In one of these the alterations were confined to the acini; no obstruction was present in the larger ducts. In the group classified clinically as celiac disease, none of whom has died, the pancreatic enzymes were all within normal range. These studies indicate that (1) interference with the formation or liberation of pancreatic enzymes plays an important rôle in the pathogenesis of pancreatic fibrosis variant of the celiac syndrome; (2) the pancreatic enzymes may be abnormally low at a time before gross obstruction to the ducts has occurred and when alterations only in the acini may be demonstrated on histologic examination; (3) the

determination of the pancreatic enzymes in the duodenal contents permits, on the basis of present evidence, the only positive differentiation between the relatively benign celiac disease, for which there is adequate medical treatment, and the more serious condition, pancreatic fibrosis.

PATHOGENESIS OF INTESTINAL RADIATION LESIONS. By Nathan B. Friedman and Shields Warren. *From the Department of Pathology, Harvard Medical School, Boston.*

Studies have been made of the development of intestinal lesions following radiation of the abdomen. Special attention was given to the pathogenesis of ulcers, and the rôle of various factors in the development of these ulcers was tested experimentally. This was done by means of surgical procedures directed at altering the normal conditions existing in a given loop of bowel in the intact animal. Interference with blood supply, immobilization by adhesions, obstruction by stenosing ligations, and deviation of the fecal stream by colostomy were carried out.

EFFECTIVE RENAL BLOOD FLOW, FILTRATION RATE AND FUNCTIONAL EXCRETORY MASS IN ESSENTIAL HYPERTENSION; DIODRAST AND INSULIN CLEARANCES. By Piero P. Foa, Ward W. Woods and Max M. Peet. *From the Department of Surgery, University of Michigan, Ann Arbor.*

Interest in the relation of renal blood flow and hypertension has been greatly stimulated by the work of Goldblatt, Page and Smith and their respective associates.

Eighteen patients with essential hypertension have been studied with respect to renal circulation. The renal blood flow was reduced in all cases, ranging as low as 145 cc. of blood per minute, as compared with the average normal blood flow of approximately 1200 cc. per minute. The filtration rate was greater than normal, indicating increased glomerular pressure. This may be a consequence of a) the increased systemic pressure, or b) a hypertonus of the efferent glomerular arterioles. Functional excretory mass (diodrast Tm) is below the normal range. The ratio between blood flow and diodrast Tm is also reduced, indicating a relative ischemia of the functioning tissue.

The patients are being studied approximately two weeks and six months after supradiaphragmatic splanchnicectomy also.

In every patient we determine the ratio of the thickness of the wall to the diameter of the lumen of the arterioles of the skeletal muscle in biopsied specimens. This will enable us to correlate the renal circulation and function with the status of the arterioles in the skeletal muscle and the appearance of the vessels of the eyegrounds.

HISTOLOGIC CHANGES IN THE SKIN OF MICE FOLLOWING RADIATION FROM MERCURY ARC. By Hugh G. Grady, Harold F. Blum, and John S. Kirby-Smith. *From the National Cancer Institute, United States Public Health Service, Bethesda, Maryland.*

Male mice of strain A were subjected to radiation from an intermediate pressure mercury arc in quartz. The dosage was accurately measured for wave lengths 3130 A and shorter by means of a titanium photocell. The in-

tensity was 4 x 10^4 ergs per second per $cm.^2$ and a total dosage of approximately 10^8 ergs per $cm.^2$ per day was given, as determined by the photocell.

Three groups of mice were exposed to this intensity for one, two and five days respectively. The histologic changes in the skin of the ears were essentially similar and were characterized by an inflammatory reaction of slow onset and evolution as well as by rapid proliferation of the epidermis. Gross erythema and microscopic evidence of an inflammatory response were not observed for 2 days following initial exposure. Hyperplasia of the epidermis progressed rapidly and well differentiated prickle cell and granular layers were formed. In 10 weeks following irradiation the epidermal changes had almost completely regressed although there was residual fibrosis of the underlying tissues.

In two other groups of similar mice radiation with 38 per cent and 23 per cent respectively, of the above intensity and dosage was continued 5 days per week throughout the course of the experiment. The early reactions of the epidermis and subjacent tissues closely paralleled those observed in the first three groups. However, at the end of 10 weeks there was no regression of epidermal hyperplasia and the inflammatory reaction was still active.

METHOD FOR TITRATING VIRULENCE OF TUBERCLE BACILLI IN MICE. By F. D. Gunn and John Sheehy. *From the Department of Pathology, Northwestern University School of Medicine, Chicago.*

Cultures of tubercle bacilli are emulsified in saline and standardized by a method previously described. Graded doses are injected intravenously into mice of an inbred strain. Death occurs in three to four weeks from pulmonary tuberculosis after the injection of large doses (0.1 to 1.0 mg.) when highly virulent strains such as the Ravenel strain are used and in five to six months with doses of 0.00001 mg. With strains of low virulence, large doses are required to produce death in mice. The results are much more consistent than those with the usual guinea pig technique.

RED BLOOD CELL VOLUME, TOTAL AND CIRCULATING, AS DETERMINED WITH RADIOACTIVE IRON. By P. F. Hahn. *From the Department of Pathology, University of Rochester School of Medicine and Dentistry.*

By repeated intravenous administration of radioactive iron to an iron-depleted anemic dog, it is possible to raise the level of erythrocytes containing the isotope to a point where the cells of this animal may be used to study the volume of red cells in other dogs.

Cells from this donor animal, containing a known amount of radioactivity, are injected into the vein of an animal under study. Blood samples are taken at various intervals thereafter, and the dilution of the radioactive erythrocytes is determined. It is thus possible to study the circulation time of the red cells and also the circulating volume of these cells. Since there is no loss of the isotope from the cells until decomposition of the cells takes place, a blood sample taken several days after the injection allows the determination of the total red cell volume as well. Plasma volume is determined simultaneously with the red cell volume.

It is found that the determination of the circulating volume of erythrocytes by the dye dilution method may be in error by 20 to 30 per cent. It

can also be demonstrated that in the normal dog the greater fraction of the red cells of the body are in active circulation at all times, since the circulating volume and total volume of red cells as estimated by this direct method are nearly the same under the conditions studied.

STUDIES OF CARTILAGE: I. SOME EFFECTS OF MEDIA pH 4-12 ON THE COMPOSITION OF CARTILAGE. By George Hass and B. Garthwaite. *From the Department of Pathology, Cornell University Medical College, New York City.*

Fresh frozen sections of infant epiphyseal cartilage in controlled lots of 50-80 mg. were extracted with buffer media, pH 4-12, and with dilute and concentrated salt solutions at neutrality for 24 hours at 5° C. Loss of weight incidental to extraction was determined and acid hydrolysates of 5-6 mg. samples of the extracted tissues were quantitatively analyzed for sulphate and reducing substances.

It was concluded that chondroitin-sulfuric acid comprises about 20 per cent of dried cartilage after extraction with 1 per cent aqueous NaCl. A small fraction of this quantity was extracted with 10 per cent aqueous $CaCl_2$ and a large fraction was extracted at pH 11, while complete extraction in the range pH 4-12 was possible only at pH 12. The complete removal of the polysaccharide in the range pH 7-12 did not account for an additional extraction of 10 per cent of the total weight of the sample, 20 per cent of the total reducing substances and 20 per cent of the total sulphate. Hence, after complete polysaccharide extraction, each section of cartilage possessed 70 per cent of the neutral extraction weight, 30 per cent of the maximum quantity of derivable reducing substances and no sulphate. The sections retained such form after all extractions that they were suitable for correlative morphological studies.

THE PRODUCTION OF GASTRIC AND DUODENAL ULCER IN VARIOUS ANIMALS BY THE INTRAMUSCULAR IMPLANTATION OF HISTAMINE IN BEESWAX. By Lyle J. Hay, David Lynn, and Owen H. Wangensteen. *From the Department of Surgery, University of Minnesota, Minneapolis.*

The experimental production of ulcer in cats by the implantation of histamine in beeswax was described previously from this laboratory by Walpole, Varco, Code and Wangensteen. Subsequently, chronic ulcers were produced similarly in the dog (Varco, Code and Wangensteen). Extension of the method to other animals has shown that chronic and perforated ulcers can be produced in a number of animals by this means. The various species' differences noted will be related. These and previously reported findings emphasize the importance of acid in the genesis of ulcer.

By this method we were able to obtain chronic peptic ulcers in the swine, guinea pig and chicken, and numerous superficial ulcerations, erosions and submucosal hemorrhages in the woodchuck, monkey, rabbit and calf.

Colored photographs and photomicrographs of the specimens were projected. Likewise, typical gastric acidity curves following single injections of histamine were shown.

QUANTITATIVE ASPECTS OF THE PHAGE-ANTIPHAGE REACTION. By A. D. Hershey and J. Bronfenbrenner. *From the Department of Bacteriology and Immunology, Washington University School of Medicine, Saint Louis.*

When coliphage and homologous rabbit antiphage of suitable concentrations are mixed in various proportions, and the mixtures are allowed to stand two or three days at low temperatures, a stable condition is reached both with respect to amount of phage neutralized and the amount of neutralizing antibody adsorbed. As the initial proportions vary from about two to 50 antibody molecules per active phage unit, the corresponding degree of neutralization, as measured by plaque counts, varies from about 10 to 95 per cent of the original phage. Somewhat larger ratios lead to complete neutralization.

The reaction is accompanied by visible aggregation and, in incompletely neutralized mixtures, the residual phage activity is found in the sediments. It appears, therefore, that the reduction of plaque counts effected by the smaller amounts of antibody may be attributed, at least in part, to aggregation of active phage. This consideration indicates that the adsorption of about 50 antibody molecules results in the neutralization of a single phage, independently of aggregation. This figure is subject to two possible sources of error, however. It is based on an antibody determination by the method of Heidelberger and Kabat, using phage-coated bacteria as antigen, which we have not adequately controlled. It also assumed that the phage preparations contained to substances other than active phage which adsorb neutralizing antibodies.

It should be noted that the result obtained here neither contradicts nor confirms an earlier finding based on statistical analysis of the neutralization curve (*J. Bact.*, 1941, **41**, 60). The latter is concerned only with those among the adsorbed molecules of antibody directly effective in neutralization.

By titration of neutral mixtures for residual neutralizing antibody, it is found that a single phage unit may adsorb up to 3,000 antibody molecules, and that amounts in excess of this remain free in the mixture. This figure does not vary appreciably with the final concentration of free antibody. It is, however, subject to the errors already mentioned.

These quantitative relations are not appreciably affected by 4-fold variations in the volume of solvent. Other attempts to demonstrate an equilibrium condition have so far led to indecisive results.

The ratio of the amount of antibody required to neutralize to the amount with which the phage can combine (1:60) is probably independent of the sources of error noted. It seems likely that the small value of this ratio may explain the superficial differences between the neutralization of phage and that of certain other viruses.

THE SIGNIFICANCE OF GLUCOSE AND NON-GLUCOSE-REDUCING SUBSTANCES IN POSTMORTEM BLOOD. By Edwin V. Hill. *From the Department of Legal Medicine, Harvard Medical School, Boston.*

The various factors which influence the rate and amount of change in the glucose and the non-glucose-reducing substances of blood after death were studied in experimental animals and in human subjects. It was found that

the glucose content of the blood in the right side of the heart differed from that in the left side. The duration of the postmortem interval, the temperature of the postmortem environment, the manner of death and the functional state of the liver and pancreas immediately prior to death were important factors in determining the rate and amount of the postmortem change.

ACUTE NECROTIZING ARTERITIS, AORTITIS, AND AURICULITIS FOLLOWING URANIUM NITRATE INJURY IN DOGS WITH ALTERED PLASMA PROTEINS. By Russell L. Holman. *From the Department of Pathology, University of North Carolina School of Medicine, Chapel Hill, N.C.*

During experiments designed to determine whether heavy metal poisoning is influenced by altering the plasma protein level, it was observed that 8 out of 10 dogs (80 per cent) subjected to alterations in their plasma proteins by plasmapheresis or plasma injections, then given uranium nitrate subcutaneously, showed well marked necrotizing arteritis when they died 8-17 days later. The lesions affected principally the elastic arteries, the commonest sites being the aorta, sinuses of Valsalva, coronary arteries, pulmonary arteries and the endocardium of the left auricle. These lesions are uniformly absent from the parenchyma of organs other than the heart and lungs.

All of the dogs which developed lesions were maintained on a standard low protein diet. Four dogs maintained on the regular kennel diet of meat scrap and subjected to the same experimental procedure failed to develop lesions. Likewise 5 dogs maintained on the standard low protein diet and receiving the same dose of uranium nitrate (5.0 mg. per kilo) but not subjected to plasmapheresis or plasma injection failed to show any arterial lesions.

Thus 3 factors seem to be essential for the production of these arterial lesions: 1. standard low protein diet, 2. plasma alteration, 3. heavy metal injury. (*See pp. 359–375 of this issue.*)

STUDIES OF THE POTENTIALITIES OF NORMAL AND PATHOLOGICAL HUMAN MONOCYTES *in Vitro*. By Ben C. Houghton. *From the Department of Pathology, Ohio State University College of Medicine, Columbus.*

Under cultural conditions the normal monocyte of the peripheral blood exhibits a wide variety of morphological patterns. In surface films during the first three days monocytes become highly phagocytic, ingesting many granulocytes and red cells. These forms become less motile, and during subsequent days increase in individual size. On the tenth day, the cytoplasm has lost its ingested material and the nucleus has become more vesicular. The stellate and fusiform character suggests a reticulum cell. At the same time many non-lymphocytic, non-phagocytic cells of the monocytic type lose the cytoplasmic and characteristic motile properties shown in the first forty-eight hours. These cells become spherical and acquire vesicular nuclei with large nucleoli. The cytoplasm develops a deeply basophilic staining property. By the tenth day, many mitotic figures may be seen among these cells and about the *Hof* of the nucleus, granulations appear. In fourteen days, cells indistinguishable from granular myelocytes, by supravital, Wright's-Giemsa or oxydase staining, are abundant. Mature granulocytes showing typical amoeboid motility reappear within three weeks. Patterns of variation and time intervals may be influenced to some degree by modification of media.

Monocytes in relatively pure cultures have been obtained for similar study from patients with chronic monocytic leukemia. These cells produce a uniform growth of "fibroblast-like" character which may be carried through numerous passages, a phenomenon which is in sharp contrast to leukemic myeloid or lymphatic cell cultivations.

The monocyte of acute monocytic leukemia, on the other hand, shows little tendency to follow the usual pattern of cyclic variations, or to change from its undifferentiated state.

MACROMOLECULAR SUBSTANCES AS DISEASE-PRODUCING AGENTS. By W. C. Hueper. *From the Warner Institute for Therapeutic Research, New York City.*

The intravenous introduction of several chemically inert, non-toxic, osmotically active, macromolecular carbohydrates, suitable as non-hematogenous blood substitutes, gives rise, when injected in excessive concentrations and amounts, to disturbances of the colloidal, macromolecular equilibrium of the plasma leading to the development of a macromolecular hematological syndrome and of an intracellular and extracellular storage of the injected material in various organs. The hematological symptom complex consists of a reduction in the number of erythrocytes and amount of hemoglobin; initial, transitory leukopenia; persistent leukocytosis; increased conglutination and sedimentation speed of erythrocytes; thrombocytopenia or thrombocytosis; impaired coagulability; normal fragility of erythrocytes; normal or moderately lengthened prothrombin time; lengthened bleeding time; hemorrhagic diathesis; decrease in fibrinogen and total plasma protein. The macromolecular material is taken up and immobilized by reticuloendothelial and endothelial cells of small and large vessels, by mobile and fixed phagocytes as well as cells of parenchymatous organs (kidney, liver, brain, suprarenal, etc.) transforming them into foam cells causing functional and anatomical disturbances. The hematological reactions and organic changes of these experimental thesauroses resemble those seen in glycogenosis, amyloidosis, lipoidoses and immunity responses.

PRODUCTION OF ELONGATED (PENCIL) ERYTHROCYTES IN CHRONIC HEMORRHAGE. By Raphael Isaacs and Leonard D. Rosenman. *From the Department of Hematology, Michael Reese Hospital, Chicago.*

Elongated (pencil) erythrocytes are present in from 0.5 to 2.0 per cent of the cells in the peripheral blood of patients who lose small quantities of blood over a long period of time. The cells have an average length of 10.72 microns (9 to 12) and a width of 3.0 microns. The sides are parallel and the ends rounded. They appeared in the peripheral blood of dogs, rats and mice subjected to repeated bleeding. Their appearance varied in different animals from 18 days to 4 weeks, and, as in man, there appeared to be no correlation between the level of the red blood cell count and the appearance of the cells. No bone marrow changes were noted which could account for the peculiar shape of the cells. No changes were noted *in vitro*. The cells are diagnostic of chronic hemorrhage.

EXPERIMENTAL POLIOMYELITIS IN GUINEA PIGS. By Claus W. Jungeblut and Murray Sanders. *From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York City.*

In previous experiments the mouse strain of SK poliomyelitis virus possessed no demonstrable pathogenicity for guinea pigs. Continued mouse transfers resulted in an increase of virulence from 1:1 million to 1:1 billion dilution activity. When examined again at the 70th mouse passage, the murine virus proved capable of paralyzing guinea pigs.

Approximately 90 per cent of the infected guinea pigs develop flaccid paralysis of the extremities following intracerebral injection with murine virus; the disease is also transmissible by feeding. The virus passes through all grades of Berkefeld filters. It may be recovered from brain or cord of paralyzed guinea pigs but not from blood; animals fed the virus discharge it in their feces.

Paralyzed guinea pigs present a typical poliomyelitic process in the spinal cord with perivascular infiltration, ganglion cell necrosis, and marked neuronophagia. Lesions in the white matter are rare; the meninges are normal.

Guinea pig passage virus is completely inactivated by guinea pig convalescent serum, antimurine rabbit immune serum and SK monkey convalescent serum; conversely, guinea pig convalescent serum neutralizes SK monkey virus. The serological evidence therefore suggests the identity of the virus in its three hosts, *i.e.*, monkey, mouse and guinea pig.

In the first guinea pig passages the incubation period was short (2 to 4 days); a gradual lengthening (7 to 21 days) occurred with subsequent transfers. Two attempts to maintain continuous serial passage in guinea pigs came to an abrupt end with the 5th and 6th passages, respectively. While active in guinea pigs, the infectious agent could easily be returned to mice and cotton rats but not to rhesus monkeys. When its virulence diminished for guinea pigs and mice, injection of a rhesus monkey with the cord of a paralyzed guinea pig produced classical poliomyelitis. It appears that a complete cycle of pathogenicity has been established for the SK strain from the monkey to cotton rats, white mice, guinea pigs and back to the monkey.

EVIDENCE OF SEROLOGICAL HETEROGENEITY OF POLYVALENT "PURE LINE" BACTERIOPHAGE. By G. M. Kalmanson and J. Bronfenbrenner. *From the Department of Bacteriology and Immunology, Washington University School of Medicine, Saint Louis.*

It is well known that a "pure line" phage may possess true polyvalence, that is, it may act equally well on several more or less related organisms, regardless of which organism was used for its propagation. It has been found earlier that such a phage (PC), acting on *B. coli, B. shiga* and *B. flexner,* is heterogeneous in the sense that by means of heat inactivation it was possible to destroy the valence for *Shiga* and *Flexner* and secure a weakly active "monovalent" phage for *B. coli.* In the present investigation we were interested in ascertaining whether the neutralizing antiserum prepared against a highly purified preparation of this polyvalent phage, and devoid of all demonstrable antibacterial agglutinins, would neutralize all three valences and, if it did so, whether it would be possible to detect by this procedure any heterogeneity corresponding to that previously demonstrated by heat inactivation.

The results of these experiments indicated that *Shiga* and *Flexner* valences may be completely neutralized by this serum before that for *coli*. When the resulting "monovalent" phage is propagated on *B. coli*, however, it immediately regains its polyvalence (*Shiga* and *Flexner*), as was found to be the case also with the "monovalent" phage secured by heat inactivation.

Statistical analysis of experimental data indicated that the observed difference in the rates of neutralization is not due to a difference in the number of antibody molecules required to neutralize one lytic unit of each valence. Therefore, the difference in the observed rates of neutralization seems to be qualitative rather than quantitative in nature.

THE RATE OF DISAPPEARANCE OF THE EASTERN EQUINE ENCEPHALOMYELITIS VIRUS FROM THE BLOOD OF RABBITS FOLLOWING THE INJECTION OF INDIA INK. By J. E. Kempf, M. E. Pierce and M. H. Soule. *From the Department of Bacteriology, University of Michigan, Ann Arbor.*

In series of 5 experiments, 19 rabbits were injected daily by the intraperitoneal and intravenous routes with a suspension of 7 per cent India ink (Higgins) in 0.85 per cent saline solution in an attempt to block the reticuloendothelial system. The efficiency of the procedure was determined on the third day by estimating colorimetrically the amount of Congo red in the serum one hour after injection. The removal of the virus of EEE from the blood stream following its intravenous injection into these animals and 21 controls was observed as follows: Blood was withdrawn from the ear vein at definite intervals and the presence or absence of virus was detected by mouse injection.

It was found that the rate of disappearance of virus from the blood was the same in the partially "blocked" animals as in the controls. However, the mortality rate from encephalomyelitis in the "blocked" rabbit was 94.7 per cent, while in the controls it was 33.3 per cent.

DISTINCT TYPES OF ANTIBODY IN THE BLOOD OF RABBITS CARRYING THE TRANSPLANTED V2 CARCINOMA. By John G. Kidd and William F. Friedewald. *From the Laboratories of The Rockefeller Institute for Medical Research, New York City.*

Experiments already reported have shown that rabbits carrying the transplanted V2 carcinoma—a squamous cell carcinoma derived originally from a virus-induced papilloma—regularly develop in their blood an antibody capable of neutralizing *in vitro* the papilloma virus with which the growth was initiated, and capable also of fixing complement in mixture with it (*J. Exp. Med.*, 1940, **71**, 813). Further studies have now shown that virus-neutralizing and complement-fixing titers of sera procured from rabbits carrying the V2 carcinoma invariably parallel one another, and that the antibody responsible for the two reactions can be readily absorbed from the sera upon admixture with the papilloma virus. From the findings it appears certain that the antibody is directed against the papilloma virus *per se* and that it is identical with the antiviral antibody present in the blood of rabbits carrying benign virus-induced papillomas and with that present in the blood of rabbits injected with purified papilloma virus (*J. Exp. Med.*, 1940, **72**, 531).

In further serum tests carried out by the methods that disclosed a dis-

tinctive substance in the Brown-Pearce tumor (*J. Exp. Med.*, 1940, 71, 335), an antibody wholly distinct from the antiviral antibody just described has been encountered in the blood of rabbits carrying the V2 carcinoma. Its properties and affinities will be reported.

STUDIES ON THE INTRAVENOUS ADMINISTRATION OF BOVINE PLASMA TO MAN. By A. J. Kremen, H. Hall, H. Koschnitzke, B. Stevens and O. H. Wangensteen. *From the Department of Surgery, University of Minnesota, Minneapolis.*

The possibility of administering bovine plasma intravenously to man was described previously by Wangensteen, Hall, Kremen, and Stevens. It is proposed to report here on extended observations made since that time with reference to (1) the efficacy of the method, and (2) nitrogen balance studies and (3) effect of agglutinin adsorption with human red blood cells on the incidence of reactions.

GASTRIC ACIDITY AFTER VARIOUS TYPES OF OPERATION FOR ULCER IN MAN. By Bernard Lannin and Owen H. Wangensteen. *From the Department of Surgery, University of Minnesota, Minneapolis.*

Achlorhydria to histamine stimulation has been set up as a desirable eventuality after operation for ulcer in man. In order to evaluate the effect of operative procedure on gastric acidity, seven types of operations have been done: (1) Anastomotic procedures, (2) Antral resection with termino-lateral gastro-jejunostomy, (3) Extensive gastric resection including excision of antrum and pylorus with termino-lateral gastro-jejunostomy, (4) Finsterer exclusion operation (same as 3, but leaving pylorus and antrum intact), (5) Small gastric resection with complete intragastric regurgitation, (6) Extensive excision of acid secreting area, and (7) Same as 6 with addition of gastro-jejunostomy.

The effect of these various operations upon gastric acidity was discussed.

THE PATHOGENESIS AND PATHOLOGY OF EXPERIMENTAL TYPE I PNEUMOCOCCUS PNEUMONIA IN THE MONKEY. By Clayton G. Loosli. *From the Department of Medicine, University of Chicago.*

Fourteen healthy monkeys were infected intrabronchially with Type I pneumococci suspended in starch and killed at increasing intervals of time within 7 days after inoculation—six were killed within the first 24 hours, seven within the next 72 hours, and one at 7 days. Lobar consolidation began at the site of inoculation and progressed in a contiguous manner until complete at 24 hours. During this time the infection was confined principally to the alveolar and bronchial air spaces. The pneumococci spread through the lobe from the site of inoculation by extension of the infected edema fluid directly from alveolus to alveolus, through the pores of Kohn, and from bronchiole to bronchiole, by repeated aspiration as the result of breathing and coughing. The cellular exudate enters the alveoli through the capillaries in the alveolar walls, and changes during the first 72 hours from one of polymorphonuclear leukocytes to one of mononuclear macrophages, in spite of early persistent bacteremias. The mononuclear macrophages arise principally from the hypertrophy of hematogenous lymphocytes and monocytes, which migrate early into the exudate, and to a lesser extent, from the

septal cells in the alveolar walls. Interstitial involvement becomes pronounced only after consolidation is complete and is associated with complications such as septicemia, and not with the mechanism which brings about consolidation.

EXPERIMENTAL SQUAMOUS CELL CARCINOMA OF THE FORESTOMACH IN MICE AND THE METHOD OF INDUCTION BY ORAL ADMINISTRATION OF CARCINOGEN. By Egon Lorenz and Harold L. Stewart. *From the National Cancer Institute, United States Public Health Service, Bethesda, Maryland.*

When an olive oil emulsion containing either 20-methylcholanthrene or 1, 2, 5, 6 dibenzanthracene is administered to different strains of mice instead of drinking water, the lesion regularly observed in the gastrointestinal tract is adenocarcinoma of the small intestine; only in rare cases squamous cell carcinoma of the forestomach was induced. Squamous cell carcinomas of the forestomach can be induced in a high percentage of animals by stabilizing the olive oil emulsion with the dioctylester of sodium sulfosuccinate and in still higher percentage by substituting mineral oil emulsion for the olive oil emulsion. Data was given as to the composition of these emulsions, the amount of emulsion consumed by the animals, and the amounts necessary for the induction of the gastric tumors. The mechanism of the induction of these tumors was discussed.

EPITHELIOMA OF LIP IN CATFISH. I. PATHOLOGY OF SPONTANEOUS TUMORS. II. GROWTH OF INTRACORNEAL TRANSPLANTS. By Balduin Lucké and H. Schlumberger. *From the Laboratory of Pathology, School of Medicine, University of Pennsylvania, Philadelphia.*

Catfish from streams near Philadelphia have been found to be commonly afflicted with epithelioma. The tumors usually occur as large, red, fleshy masses on the lips or dental plates; sometimes they become so massive as to prevent closure of the mouth. In approximately one-third of the cases, secondary tumors develop on that part of the opposite lip in direct contact with the primary growth. Histologically, the neoplasms consist of epithelial cells, often in papillary arrangement; the larger tumors commonly extend downward, invade adjacent tissues, and push into vessels in which tumor emboli are frequently found. Experimentally, we have transmitted the tumor to fish of the same species by implanting it between the layers of the cornea (which in catfish are readily separable). Here the growth of the transplants has been followed by periodic examination of the living tumors with the slit lamp microscope. It was thus learned that this cancer develops according to a definite structural pattern, namely, in the form of undifferentiated membranes which gradually thicken and become compact. In this manner growth continues until the tumor fills the cornea.

HYPERTHERMIA AND DEATH CAUSED BY A CLOSED DUODENO-JEJUNAL LOOP AFTER GASTRIC RESECTION; PREVENTION OF THE COMPLICATION; EXPERIMENTAL REPRODUCTIONS OF THE SYNDROME IN THE DOG. By David Lynn, Lyle J. Hay and Owen H. Wangensteen. *From the Department of Surgery, University of Minnesota, Minneapolis.*

Hyperthermia and death have been observed in man, as complications of gastric resection, as well as of gastro-jejunostomy performed for high-grade

obstruction of the duodenum. The paucity of significant peritoneal pathological findings at autopsy has caused such deaths to be attributed usually to atelectasis or pneumonia. Mindful of the suggestion of C. A. Dragstedt and his associates that relatively low sustained intraluminal pressures in the duodenum may compromise the viability of the bowel, a comparable situation was established in the dog. Hyperthermia was a frequent complication. A satisfactory method of preventing the occurrence of this complication in man after gastric resection was described.

HYPOPROTEINEMIA TREATED BY PROTEIN DIGESTS BY VEIN. By Sidney C. Madden. *From the Department of Pathology, University of Rochester School of Medicine and Dentistry.*

Hypoproteinemia is produced in dogs by plasmapheresis. A casein digest given by vein will maintain such a hypoproteinemic dog in nitrogen balance and weight equilibrium for many weeks provided that adequate non-protein requirements are given by mouth or otherwise. Such a dog getting intravenous digest regains his normal plasma protein concentration when plasmapheresis is discontinued.

THE EFFECT OF HEPATECTOMY, AND ABDOMINAL EVISCERATION WITH AND WITHOUT HEPATECTOMY ON THE SERUM PHOSPHATASE OF THE DOG. By Stephen Maddock, Harry C. Trimble, Dorothy Jensen and William Appleby. *From the Boston City Hospital.*

In a series of dogs it has been found that total hepatectomy results in a rise of serum phosphatase to the neighborhood of 50 Bodansky units during the course of the experiment. The increase in serum phosphatase is not due to anesthesia, nor can it be accounted for on the basis of injected glucose, since glucose given hourly in amounts comparable to those needed to prevent hypoglycemia in the liverless dog causes no rise in phosphatase.

When all the abdominal viscera including the liver and kidneys are removed there is no increase in serum phosphatase in the early postoperative hours and only a slight terminal rise to the vicinity of 10 Bodansky units.

The removal of the entire gastro-intestinal tract, spleen and pancreas, leaving the kidneys and liver intact, causes no rise in serum phosphatase.

These results are interpreted as meaning that phosphatase coming from the intestines and possibly the kidneys and bone also, is utilized by the liver for the dephosphorylation of hexose-1-monophosphate (Cori ester). In the absence of the liver the enzyme accumulates in the blood.

The rise in phosphatase following hepatectomy is much more rapid than that found after common bile duct ligation since it ordinarily requires 5 to 7 days for the serum of these animals to reach a value of 50 Bodansky units. This gives some conception of the magnitude of the rôle of the liver in the utilization of phosphatase. These experiments suggest a method for the future study of the origin of phosphatase in the animal body, and a means of determining whether more than one type of alkaline phosphatase exists.

SEX HORMONES AND LYMPHOMATOSIS OF FOWLS. By David Marine and Samuel H. Rosen. *From the Laboratory Division, Montefiore Hospital, New York City.*

We have previously reported upon the occurrence of lymphomatosis in 24 of 56 White Leghorn capons and its absence in 61 slips of the same group—

19 of which were older than the youngest bird that developed the disease. This observation suggested that the presence of a functionally active fragment of testis may have inhibited the development of the disease—possibly because of the production of sex hormones.

The present experiment includes 41 White Leghorn male castrates, divided into 3 groups as follows: 1) 12 birds, each receiving 1 mg. of estradiol dipropionate, weekly; 2) 12, each receiving testosterone dipropionate in variable amounts twice weekly; and 3) 17 controls. Injections of these hormones were started on June 18, 1940, and they are still being given to the survivors.

To date (January 15), 4 or 33⅓ per cent of the androgen group, 7 or 58 per cent of the estrogen group and 4 or 23.5 per cent of the control group have died of lymphomatosis. The youngest (receiving estradiol) was 201 days old and the oldest to date, 319 days. Routine biopsies have shown that the liver may be transformed from a normal appearing organ to a huge reddish gray organ in 15 days.

In only one of the 15 cases was a fragment of testis found at autopsy—confirming our observation in the first series.

Anatomically the cases may be grouped as 1) diffuse hepatorenal (big liver disease) type—8 cases; 2) lymphosarcoma—4 cases; 3) lymphoma of the bursa of Fabricius—2 cases; and 4) neurolymphomatosis—1 case. The incidence of lymphomatosis in the androgen group may not be significant since the dose of androgen during the first 4 months of the experiment varied from excessive to a submaintenance level. The occurrence of lymphomatosis in 58 per cent of the estrogen group as opposed to 23.5 per cent of the control group suggests that the induced sex hormone imbalance may have been a factor in stimulating or activating the agent responsible for overgrowth of lymphoblastic tissue.

PERIPHERAL VASCULAR REACTIONS IN ANAPHYLACTIC SHOCK OF THE MOUSE. By Philip D. McMaster. *From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*

The vascular reactions in the ear of the mouse have been studied under high power during anaphylactic shock produced by a highly colored antigen, the proteins of horse serum coupled to an azo dye, T-1824, by diazotization.

After the antigen has been injected into the blood, local or general constriction of veins often occurs either before or after the arterial spasm, and sometimes together with it. If venous spasm precedes arterial spasm, the capillary bed becomes choked with cells; if it occurs later, the tissues become bloodless. No constriction of capillaries has yet been observed; the capillary behavior seemed to follow passively the changes in the large vessels.

Peripheral vascular spasm occurs while the carotid blood pressure is high and none of the colored antigen can be perceived in the vessel endothelium. Following the vascular reaction the blood pressure falls. The lymphatics undergo no change nor is there any in the interstitial pressure.

If the circulation of one ear is obstructed during anaphylactic shock, no vascular spasm occurs in it. Release of the obstruction, during the animal's recovery, results in belated constriction of the blood vessels, although now the vessels in the other ear are dilated and the general blood pressure is low.

The vascular reactions in the ears are not the consequence of nervous stimuli but are local in origin, and are uninfluenced by the blood pressure.

PULMONARY ARTERY TO LEFT AURICLE ANASTOMOSIS WITH HYPERTROPHIC OSTEOARTHROPATHY. By M. Mendlowitz and A. Leslie. *From the Laboratories of the Mount Sinai Hospital, New York City.*

By a special technique the pulmonary artery was successfully anastomosed to the left auricle in four dogs. The cardiac output (Fick method), blood volume, arterial and venous blood pressures, ether and cyanide circulation times were measured before and one or more times after operation in three of these dogs. The per cent shunt was also calculated. Two dogs died three months and three weeks, respectively, after operation of intercurrent infection (pneumonia). The first of these dogs had a 46 per cent shunt and developed a moderate secondary polycythemia and a systemic cardiac output in excess of the preoperative. The blood flow through the lungs was normal. The cyanide circulation time and the arterial blood pressure were decreased but the oxygen consumption and all the other determinations remained unchanged. The other dog had an obviously large shunt (cyanosis) but died before postoperative studies could be carried out. The remaining two dogs are still alive 9 and 7 months, respectively, after operation and are in good condition. In the former there is a 13 per cent shunt, a somewhat decreased cyanide circulation time, an excessive systemic cardiac output but no other changes. In the latter there is a 17 per cent shunt, a short cyanide circulation time, and excessive systemic cardiac output and bone changes indistinguishable by X-ray from hypertrophic osteoarthropathy. The only consistent circulatory change and probable cause for this bone condition is a systemic cardiac output in excess of tissue needs.

ON THE MECHANISM OF ENHANCED DIABETES WITH INFLAMMATION. By Valy Menkin. *From the Department of Pathology, Harvard Medical School, Boston.*

A study has been undertaken in an effort to determine the factors concerned in influencing the state of diabetes with inflammation while at the same time the inflammatory reaction appears concomitantly to be intensified. In brief, it has been found that in the depancreatized dog with inflammation there is an enhancement in the degree of diabetes as indicated by the marked rise in blood sugar. There is likewise a concomitant increased proteolysis at the site of inflammation. This is indicated by the rise in urea, non-protein nitrogen, and amino acid nitrogen of exudates. The rise in the concentration of sugar and lactic acid of exudates seem to be referable, at least in part, to the increased proteolysis favoring thus, by deamination, the conversion of part of the protein molecule to glucose. The conversion of protein or amino acids to glucose and lactic acid is known to occur in the diabetic animal as described in the earlier work of Lusk and of others. Tissue injury is essentially the result of proteolytic processes. An inflamed area is a focus of proteolysis. The hypothesis advanced by the writer is that such foci manifest an intensified degree of proteolysis in the diabetic organism. Thus the enhanced local proteolysis in a diabetic animal, either incapable of burning its own glucose or else producing an over-abundance of this substance, would both intensify tissue damage by protein degradation and at the same time favor local gluconeogenesis. Furthermore, studies also indicate that insulin administration inhibits not only the more severe manifestation of diabetes, but also the enhanced proteolysis at the site of inflammation.

The available evidences, therefore, support the view that the mechanism of enhanced diabetes with concomitant inflammation might well be referable primarily to an increased local proteolysis in the inflamed area, favoring a combined picture of increased tissue damage with a corresponding elevation in glucose formation; the glucose, in turn, gradually diffuses into the systemic circulation.

LESION PRODUCED IN THE BONE MARROW BY INJECTION OF SPECIFIC ANTIBODIES. By Anderson Nettleship. *From the Department of Pathology, Union University, Albany Medical College.*

Antibodies capable of producing widespread changes in the bone marrow were obtained by injecting extracts of bone marrow or leukocytes into an heterologous species. These extracts were prepared by grinding rabbit bone marrow or leukocytes, and following a preliminary fat extraction the protein was precipitated with abundant cold acetone. The water soluble precipitate was injected twice weekly into guinea pigs for a period of eight weeks. Ten days after the last injection the guinea pigs were bled out and their serum pooled. Five to ten milliliters of this serum was injected intravenously into rabbits. In acute experiments the peripheral blood and bone marrow were studied prior to and following a single injection. In chronic experiments the same technique was used, following multiple injections, over a period of six weeks. The results showed a dramatic drop in circulating polymorphonuclear leukocytes within ten minutes following the injection. This persisted for as long as twenty-four hours. Sections of bone marrow taken within seventy-two hours following the injection showed fresh necrosis and hemorrhage in small irregular shaped areas throughout the bone marrow cavity. The chronic preparations showed not only areas of necrosis but also areas of marked hyperplasia. There were also many cysts and areas of heavy fibroblastic proliferation. These latter bear a striking resemblance to osteitis fibrosa as seen in humans.

INFLUENCE OF BREEDING ON SUSCEPTIBILITY OF WHITE RATS TO TRANSPLANTATION OF A METHYLCHOLANTHRENE SARCOMA. By J. Lowell Orbison, H. A. Davenport and Frank B. Queen. *From the Department of Anatomy and the Patterson Laboratory for Cancer Research, Northwestern University Medical School, Chicago.*

A spindle cell rat sarcoma (our No. R 86), which was induced by methylcholanthrene, failed to grow in some litters, while nearly all implants grew in other litters. Assuming this to be a manifestation of heredity, breeding experiments were begun to attempt a purification of the factors responsible. The procedure used to separate the resistant and susceptible lines was a selection of pairs which had been tested by transplanting when they were about two months old. As the two lines were developed, the value of a particular mating was determined by transplanting the entire litter of offspring, and using only those individuals for further breeding which produced or were part of completely resistant or susceptible litters. Susceptible pairs were saved for breeding after total extirpation of the growing tumors. Between September 1, 1940 and December 20, 1940, seventeen litters of each line have been transplanted with the tumor to test their responses. The seventeen

litters of susceptibles contained 95 rats, and of these 94 showed one or more progressively growing takes (99 per cent). Each rat received four implants except 10 which received only two. Of the 360 implants made, 343 grew (95 per cent). The seventeen resistant litters contained 110 rats, and of these 40 showed one or more progressively growing takes (36 per cent). Each rat received four implants except 19 which received only two. Of the 402 implants made, 82 grew (20 per cent). The tumor had been stabilized previously by passing it through 15 transplant generations. Although the experiment is not completed, the selection seems to have produced two hereditary lines which exhibited marked difference in susceptibility to tumor implantation. A recent complication has arisen in that most of the resistant litters have failed to reproduce.

EFFECTS OF DIETARY YEAST ON DEVELOPING AND HEALING CARBON TETRACHLORIDE CIRRHOSIS IN THE RAT. By Joseph Post, David P. Earle, Jr. and Joseph Victor. *From the Research Service, First Division, Welfare Hospital, Department of Hospitals, City of New York, and the Department of Medicine, College of Physicians and Surgeons, Columbia University.*

The influence of feeding various amounts of yeast on CCl_4 cirrhosis was studied. When the food intake was restricted to the same level in all animals, there was no difference in the development of cirrhosis between rats fed large amounts or minimal amounts of yeast needed for normal growth. Likewise the healing of cirrhosis was the same in both groups. However, when the food intake was uncontrolled, cirrhosis was more severe in rats fed yeast in amounts below that necessary for normal growth.

CCl_4 injections markedly inhibited the growth of rats regardless of the yeast intake. The poor growth of rats developing or recovering from CCl_4 cirrhosis of the liver was improved by the feeding of yeast in excess of the normal rat requirements. A similar growth acceleration was not observed by feeding comparable amounts of yeast to uninjected controls. High yeast feeding exerted a limited lipotropic effect on the fatty infiltration of the liver resulting from CCl_4 administration.

THE DEVELOPMENT OF THE AGENT OF LYMPHOGRANULOMA VENEREUM IN THE YOLK SAC OF THE CHICKEN EMBRYO. By Geoffrey Rake, Helen Jones and Morris F. Shaffer. *From the Squibb Institute for Medical Research, New Brunswick, N.J.*

The agent of lymphogranuloma venereum, when introduced into the yolk sac of the developing chicken embryo, produces an infection fatal to the embryo in from 3 days to 2 weeks. Studies on the distribution of the virus by transfer to new series of embryos by the yolk sac route show that the virus is present in large quantities in the wall of the yolk sac and in the yolk itself. The development of the agent in the wall of the yolk sac has been investigated by means of histological sections and alcohol-fixed impression smears. The pathological picture consists essentially of the development, within the yolk cells, of small foci of dark-staining bodies which increase rapidly in number and, concurrently, decrease somewhat in size until the cytoplasm of the distended yolk cell is filled with small particles. These

bodies are apparently identical with the granulo-corpuscles of Miyagawa and appear to be elementary bodies of the agent. Definite correlation has been found between the development of the virus as shown by pathological preparations, and titrations of the yolk sac for infectivity.

THE LIVER GLYCOGEN CONCENTRATION FOLLOWING INTRAVENOUS GLUCOSE ADMINISTRATION AND DIET ON THE DOG AND MAN IN THE PRESENCE OF LIVER INJURY. By I. S. Ravdin, Harry M. Vars, Elizabeth Thorgood and Julius Schultz. *From the Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia.*

It is generally believed that high concentrations of hepatic glycogen protect the liver from many hepatotoxic agents. Regardless of the validity of this impression, it is also widely believed that glucose administered intravenously is the best method of increasing hepatic glycogen. This is untenable except for brief periods of time, since it disregards the calorific requirements of man or animal. We have studied this in the dog in the presence of common duct obstruction.

During voluntary feeding animals lost hepatic glycogen and increased liver lipid concentration. When in addition glucose was administered intravenously, the glycogen concentration was maintained and the liver lipid concentration was decreased. Where appetite was stimulated and the dogs received glucose intravenously, the liver glycogen concentration increased by 84 per cent and the lipid concentration decreased by 25 per cent. Following forced feeding (88 calories per kilogram of body weight per day) the liver glycogen concentration increased by 236 per cent and the lipid concentration decreased by 73 per cent. With only a 5 per cent solution of glucose intravenously (50 cc. per kilogram of body weight per day) the animal lost 50 per cent of the original liver glycogen concentration.

In man similar data have been obtained. The data from man and the dog suggest that if large accumulations of glycogen are desirable these must be obtained by the oral administration of foodstuffs, for such stores cannot be maintained unless the energy requirements are being met.

THE DETECTION OF MILD OR SUBCLINICAL SCURVY. By James F. Rinehart. *From the Department of Pathology and Medicine, University of California Medical School, San Francisco.*

This study is directed at the detection of mild or subclinical scurvy. It is based upon the correlation of many observations made during the past 5 years.

Fasting blood ascorbic acid concentrations have been correlated with curves of plasma concentration and urinary excretion following the ingestion of a large dose of ascorbic acid (15 mg. per kilogram). These data indicate that the character of the curve and the degree of undersaturation can be reasonably accurately predicted from the fasting blood concentration. Plasma ascorbic acid values of 0 to 0.1 mg. per cent usually indicate marked tissue depletion. The curves are usually flat and there is no urinary excretion. In a number of such cases where it has been possible to determine accurately the ascorbic acid deficit it has been found to be in the range of 3 to 4 grams. This is the approximate deficit existing in clinical scurvy. While

fasting ascorbic acid concentrations of 0.1 to 0.2 mg. per cent represent distinctly less marked depletion, evidence of the type set forth below, indicates that they are probably clinically significant.

The absolute diagnosis of subclinical scurvy should be based upon some objective or clearly defined subjective change resulting from administration of vitamin C in cases suspected of deficiency. Two useful objective tests are determination of the capillary resistance and study of erythropoietic responses. Using these criteria it is shown that plasma ascorbic acid levels below 0.2 mg. per cent are usually indicative of clinically significant deficiency. Values of 0.3 to 0.5 mg. per cent probably do not indicate significant deficiency even though the saturation levels are considerably higher, *ie.*, 1.0 to 1.4 mg. per cent.

COMPARISON OF HEMOGLOBIN PRODUCTION IN EXPERIMENTAL ANEMIA OF SEVERE AND MODERATE DEGREE. By F. S. Robscheit-Robbins. *From the Department of Pathology, University of Rochester School of Medicine and Dentistry.*

These studies concern an experimental hemorrhage-anemia of varying degrees of severity in dogs. The effect of a variety of diet factors on hemoglobin production is determined in an anemia of severe type as represented by hemoglobin levels of approximately 6 gm. and in anemia of more moderate degree with hemoglobin levels approximating 10 gm. The response to diet factors as concerns hemoglobin production decreases as the anemia becomes less severe. A comparison of results obtained was made.

STUDIES ON THE RELATION OF "NEUROTROPIC" STREPTOCOCCI TO POLIOMYELITIS AND ITS VIRUS. By Edward C. Rosenow. *From the Division of Experimental Bacteriology, The Mayo Foundation, Rochester, Minnesota.*

The studies here reported represent attempts to find the probable nature of the relation of the more or less characteristic cultivatable bacteria which are associated commonly with virus diseases. The streptococci were isolated from poliomyelitic and other material by the use of mediums especially favorable for growth of streptococci. Pure cultures of streptococci, far removed from virus, were grown in autoclaved infantile tissue consisting of the contents and shells of nineteen-day hatching eggs passed through a meat chopper (one part) and distilled water (seven parts). After growth for a variable time in this medium, extremely small pleomorphic forms appeared and a virus phase of the streptococcus sometimes developed. Virus "takes" were obtained first in mice and then after successive passages, in monkeys. The symptoms and lesions at first are chiefly encephalitic but after successive passage through monkeys, they become typical of poliomyelitis. The experimentally produced virus strains are filtrable and transmissible in series, and induce characteristic disease in extremely high dilutions. Monkeys that have recovered from natural virus are resistant to the experimental virus. Neurotropic streptococci were isolated with the same difficulty as from "natural" virus. Only strains that have neurotropic cataphoretic velocity, virulence and serologic properties, regardless of original source, have yielded virus.

STUDIES IN MELANURIA. By Stephen Rothman. *From the University of Chicago.*

In 1935 Linnel and Raper showed that the chromogen in the urine of patients with malignant melanotic tumors is a simple derivative of 5-6 dihydroxy-indole which is also formed in the tyrosine-tyrosinase reaction by intramolecular rearrangement of catechol derivatives.

In clinical medicine the only qualitative chemical reaction used for detection of chromogens in melanuria is the darkening of the urine by addition of ferric chloride. This reaction is neither very sensitive nor specific enough, and in the case of dark urines its result is often doubtful. The findings of Linnel and Raper have been utilized in a case of melanuria (Service of Dr. G. F. Dick) by applying a series of sensitive qualitative color reactions which are characteristic for the chromogens (Thormählen, Ehrlich, nitroso-indol reaction) and to differentiate them from other indole compounds occurring in the urine such as indole and indican. Simple fractioning procedures were described for accumulation of the chromogen in order to increase the sensitivity of its qualitative color reactions.

EVIDENCE FOR DIFFERENT MECHANISMS IN CARCINOGENESIS BY HYDROCARBONS AND ULTRAVIOLET IRRADIATION. By H. P. Rusch and B. E. Kline. *From the McArdle Memorial Laboratory, University of Wisconsin, Madison.*

An attempt was made to determine whether the mechanism of carcinogenesis induced by ultraviolet irradiation is similar to that caused by the hydrocarbons. Ultraviolet irradiation of albino mice for thirty minutes daily over a period of four to five months resulted in a high percentage of ear cancers. The concurrent application of various carcinogenic hydrocarbons to the ears did not accelerate tumor formation. In other experiments mice were irradiated for periods of two to three months and hydrocarbons were applied to the irradiated parts for two months either preceding or following the irradiation. Each of these treatments was by itself just sub-carcinogenic. If the two treatments had supplemented one another, as the hydrocarbons do, cancers should have developed. However, no synergistic effect was observed and hence it is unlikely that the exposure of living tissue to ultraviolet irradiation results in the formation of carcinogens similar to the known hydrocarbons. Benzene extracts of irradiated skin were non-carcinogenic.

THE EFFECT OF INSULIN AND INSULIN PLUS THYROXIN ON THE METASTASIS OF THE BROWN-PEARCE EPITHELIOMA. By R. Ryer and J. R. Murlin. *From the Department of Vital Economics, University of Rochester.*

Six experiments were conducted. Three are concerned with the administration of insulin alone, two with insulin and thyroxin administered together, and one with thyroxin alone.

Young *inbred* New Zealand white bucks were inoculated with a sterile saline, cell suspension of the tumor. Death time was equalized by paired killing. All tumor was removed, weighed and separated into primary and individual metastasis in the last four experiments.

In experiments with insulin alone 84 animals were used (46 control, 38 treated). In summary (expressing insulin-treated as per cent of control):

primary size 79 per cent; total metastatic weight 65 per cent; average metastatic weight 60 per cent; per cent mortality from carcinomatosis 50 per cent; animals with metastatic involvements 80 per cent; degree of metastasis 50 per cent. The differences are significant statistically.

Two experiments using insulin and thyroxin together made use of 60 animals (35 control, 25 treated). Comparison of the two groups indicates: total weight 21 per cent; metastatic weight 11 per cent; total tumor per animal 27 per cent; number metastases per animal 16 per cent; total number of metastatic involvements 34 per cent; total number of involvements per animal 50 per cent; metastatic involvements alone per animal 45 per cent; total severity (average weight at a locus times average occurrence of that locus) 26 per cent; metastatic severity 7 per cent. The differences are highly significant statistically.

The sixth experiment involving 60 animals is still in progress.

SOLUBLE ANTIGEN IN LYMPHOGRANULOMA VENEREUM. By Morris F. Shaffer and Geoffrey Rake. *From the Squibb Institute for Medical Research, New Brunswick, N.J.*

Previous workers have concluded that the Frei test demonstrates an allergic reaction due to the presence of the elementary bodies of the virus in the material injected. Thus it has been found that (a) with certain Frei antigens high-speed centrifugation yields non-reactive supernatants and dermally active sediments; (b) the passage of such Frei antigens through filters such as Berkefeld and Seitz K greatly reduces or completely abolishes the dermal activity.

With the aid of rich suspensions of virus, propagated in the yolk-sac of the chicken embryo or in mouse-lung, we have reinvestigated the problem. We have found that centrifugation of such suspensions at 12,000 RPM results not only in the deposition of virus-containing sediment but also yields a supernate which, despite its depletion in active virus, after further passage through Seitz EK discs still retains considerable ability to elicit a positive Frei reaction and to fix complement specifically in the presence of human lymphogranulomatous serum. Attempts to filter rich yolk-sac virus suspensions through Seitz EK filters result in filtrates with an infectivity reduced usually to zero or at least by more than a millionfold but with eight to fiftyfold diminished ability to elicit Frei reactions and to fix complement specifically. For such filtrates, therefore, antigen is present not in the form of elementary bodies but in a less highly organized, presumably soluble, form.

THE L-S SOLUBLE ANTIGEN OF VACCINIA. By T. Shedlovsky and J. E. Smadel. *From the Department of Medicine, The Hospital of The Rockefeller Institute for Medical Research, New York City.*

The heat labile (L) and heat stable (S) soluble antigens of vaccinia generally occur as a complex substance (L-S), (Craigie and Wishart). Several experiments (Parker, Craigie and Wishart) suggest that occasionally the complex may be dissociated, leaving free L and free S antigens.

Appreciable amounts of pure L-S complex were obtained from dermal filtrate by physico-chemical methods. Studies on such preparations by electrophoresis and also in the ultracentrifuge revealed only one component. The solutions precipitated to equal titer with L and S antibodies.

Solutions of pure L-S antigen after heating precipitate with S antibody but not with L antibody; combination with L antibody still occurs, however, as shown in inhibition tests. L-S antigen treated with heat and dilute alkali completely loses all serological activity with L antibody and no longer precipitates with S antibody, but does inhibit it. These observations on pure L-S antigen confirm earlier results from our laboratories obtained with crude dermal filtrate.

It appears that L-S antigen is a molecular structure with two antigenically distinct parts, L and S. An hypothesis based on degradation of the S portion without change in the L portion of the molecule could explain the very occasional observations, which other workers have interpreted to indicate dissociation of the complex, into free L and S antigens.

TESTICULAR TUMORS IN MICE INJECTED WITH STILBESTROL. By Michael B. Shimkin and Hugh G. Grady. *From the National Cancer Institute, United States Public Health Service, Bethesda, Maryland.*

Male mice of strain C (Bagg albino), three months of age, received a single subcutaneous implantation of a 6 to 8 mgm. cholesterol pellet containing 25 to 50 per cent of diethyl stilbestrol. Enlargement of the testes, usually unilateral, was noted about six months later. Within eight months approximately one-third of the animals have developed primary testicular tumors. The growths were composed of broad sheets of polygonal cells and appeared locally invasive. Large quantities of yellow pigment and moderate quantities of stainable fat were present. Necrosis was a prominent feature.

PARASYMPATHETIC EFFECTS OF PHOSPHOLIPIDS AND ERYTHROCYTES. By Gregory Shwartzman, M. B. Bender and E. Wachtel. *From the Mount Sinai Hospital, New York City.*

Laked erythrocytes injected intravenously or erythrocytes hemolyzed in the blood stream produce parasympathetic effects in the cat. Preliminary studies suggest that the substance producing these effects is associated with the phospholipid of the red corpuscles. The lecithins and cephalin extracted from human red blood cells, several vegetable and animal lecithins and synthetic lecithins have the same pharmacologic properties as the corpuscles, *viz:* when injected intravenously in aqueous emulsion each constricts the completely denervated iris of the cat but not that of the dog, monkey or rabbit; each produces a drop in the blood pressure of the cat, dog, and rabbit, but very little in the monkey. The synthetic lecithin is the most potent of the phospholipids tested. The isolated guinea pig ileum contracts with small quantities of lecithin and cephalin. Egg lecithin, cholesterol and gum acacia have no miotic effects.

The parasympathetic effects of the erythrocytes, lecithins and cephalin are not due to choline because none of these substances is blocked by atropine nor is any of them potentiated by eserine. Furthermore cephalin does not contain a choline radical. Recently we tested a compound in which the choline was excluded from the synthetic lecithin variety (distesoglycerophosphoric acid). This compound is more potent than the synthetic lecithin. It is inferred that the parasympathetic effects obtained by erythrocytes are due to the phospholipid in the blood.

FATTY CHANGES IN THE GLOMERULI OF THE KIDNEYS. By James P. Simonds and Jack D. Lange. *From the Department of Pathology, Northwestern University Medical School, Chicago.*

The glomeruli were examined for fat in Sudan-stained sections of the kidneys of 37 control dogs, of 96 dogs that had been injected intravenously with minute doses of colloidal poisons (bacterial toxins) and crystalline poisons such as potassium dichromate, and of 76 humans with varying types of nephropathies. Fatty changes may occur in the glomeruli in acute infectious diseases, in acute and chronic glomerulonephritis and in nephrosclerosis. In acute infections and in glomerulonephritis the fat is most abundant in the immediate vicinity of the glomerular hilus and the afferent artery is free from fat. In nephrosclerosis the afferent arteries contain much fat which stops at the glomerular hilus. The fat in the glomeruli appears first in the periphery, the central part being involved only in those in which fat is very abundant. In the first group, it is believed that the fatty changes are due to direct injury to the cells by a toxin circulating in the blood and concentrated in the glomeruli by loss of water by filtration; in nephrosclerosis, they are believed to be the result of ischemia due to narrowing of the lumen of the afferent artery.

THE INFLUENCE OF THE CONCENTRATION OF VIRUS PARTICLES ON INFECTION WITH VACCINIA. By Douglas H. Sprunt. *From the Department of Pathology, Duke University School of Medicine, Durham, N.C.*

Experiments have been done which show that the ability of vaccinia to produce a lesion is dependent not only on the amount of virus injected but also on the volume of tissue the virus reaches shortly after injection. The larger the volume of tissue the virus comes in contact with, the greater the chance for infection. In other words, if 10 virus particles are distributed through a tissue volume of 4 cc. their chance of producing a lesion is better than if these 10 virus particles were distributed through only 2 cc.

These conclusions have been reached by the following experiments:

1. Experiments in which the volume of inoculum is varied. For example, it is shown that 10 virus particles in 0.1 cc. are no more likely to cause an infection than 5 virus particles in 0.5 cc.

2. Experiments in which the inoculum is kept localized with the estrogenic hormone. For example, if 10 virus particles are kept localized within a volume of tissue of 2 cc. their chance of producing an infection is no greater than if 5 virus particles are spread through 4 cc.

3. Experiment in which the virus particles were greatly dispersed with a spreading factor. In this experiment it is shown that the increased dispersion greatly increases the likelihood of infection.

These experiments not only show the difficulty of obtaining precise information from virus titration in animals but also emphasize again the inherent parasitic nature of viruses.

THE INFLUENCE OF ACTIVE AND INACTIVE ANTI-ANEMIC PRINCIPLES UPON THE SIZE OF THE ERYTHROCYTES OF OPOSSUMS (*Didelphis virginiana*) IN THE MATERNAL POUCH. By Joseph Stasney and Edward L. Burns. *From the Department of Pathology and Bacteriology of the Louisiana State*

University School of Medicine and the Department of Pathology of the Charity Hospital of Louisiana, New Orleans.

Because of the similarity of erythrocytes in the mammalian fetus and in patients with pernicious anemia, attempts were made to influence the rate of maturation of the fetal red blood cells by administering anti-anemic principles. The opossum (*Didelphis virginiana*) was found to be a satisfactory species for this purpose because intra-uterine life lasts only about ten days, after which the immature young live and are easily accessible in the maternal pouch for 90 days.

In 117 immature opossums in the maternal pouch the mean maximal diameter of the erythrocytes was greater and the mean corpuscular volume larger than these respective values in ten adult animals of the same species. The direct injection of either normal human gastric juice or of a concentrated solution of liver extract into 38 immature pouch opossums significantly reduced the mean maximal diameter and the mean corpuscular volume of the red blood cells as compared with 27 untreated litter mate controls. These changes were effected through a relative increase in the number of smaller erythrocytes. The direct injection of either inactivated normal human gastric juice or of an inactivated concentrated solution of liver extract into 26 immature pouch opossums did not significantly reduce the mean maximal diameter and the mean corpuscular volume of the red blood cells as compared with 18 untreated litter mate controls. Gastric juice obtained from a patient with Addisonian pernicious anemia also failed to produce a significant reduction in the mean maximal diameter and in the mean corpuscular volume of the red blood cells of five immature pouch opossums as compared with three untreated litter mate controls.

THE NON-SAPONIFIABLE LIPID FRACTION OF LIVER FROM CANCEROUS AND NON-CANCEROUS PERSONS. By Paul E. Steiner. *From the Department of Pathology, University of Chicago.*

The amount of total non-saponifiable lipids recovered from the livers of thirty-three persons having cancer, eleven with various non-neoplastic diseases, and eight with cirrhosis of the liver, show no significant differences. The individual difference is enormous, varying from 0.32 per cent to 2.78 per cent, and it is not correlated with the type of tumor, the location of the primary tumor, or the amount of fatty change (fatty degeneration and fat infiltration) visible on microscopical sections. The fraction is of interest because it has cancerogenic properties.

MORBID ANATOMY OF INDUCED TUMORS OF THE FORESTOMACH IN MICE FOLLOWING ORAL ADMINISTRATION OF CARCINOGEN. By Harold L. Stewart and Egon Lorenz. *From the National Cancer Institute, United States Public Health Service, Bethesda, Maryland.*

Mice which have received 20-methylcholanthrene orally as described in another abstract (see Egon Lorenz and Harold L. Stewart, "Experimental squamous cell carcinoma of the forestomach in mice and the method of induction by oral administration of carcinogen") showed the following lesions of the forestomach, singly or in combination: hyperkeratosis and hyperplasia of the mucosa, single or multiple papillomas, and squamous cell carcinoma.

In addition a number of the animals with changes in the stomach had adenocarcinoma of the small intestine. The mucosa of the pyloric chamber of the stomach was not involved except in certain cases in which the neoplasm in the cardiac chamber overhung somewhat the limiting ridge separating the two gastric chambers. The carcinomas of the forestomach were all of the squamous cell type. They extended through all coats of the gastric wall, formed nodular masses on the peritoneal surface and showed infiltration of or metastases to the following: mesentery, pancreas, mesenteric lymph nodes, genital omentum, spleen, diaphragm, lung, chest wall and posterior abdominal wall. Lantern slides illustrating the gross and microscopic features of these lesions were demonstrated.

LYMPH PROTHROMBIN. By S. A. Walker and K. M. Brinkhous. *From the Department of Pathology, State University of Iowa College of Medicine, Iowa City.*

In an effort to obtain further knowledge concerning the distribution of prothrombin in the body, its concentration in lymph collected from various sites was studied. Twenty-one normal dogs were used. The prothrombin content of thoracic duct, femoral and hepatic lymph was determined by the method of Warner, Brinkhous and Smith. It was found that the prothrombin content of liver lymph was nearly equal to that of dog plasma, while the prothrombin content of femoral lymph was less than one-tenth that of plasma. The prothrombin values for thoracic duct lymph varied widely, averaging about 50 per cent of the dog's plasma prothrombin. Fibrinogen determinations done at the same time on thoracic duct lymph averaged 51 per cent of the plasma fibrinogen. These results as a whole correspond closely to those which have been obtained for total proteins in lymph.

THE BEHAVIOR OF CELLULAR PROTEINASES (CATHEPSINS) IN EXPERIMENTAL TUBERCULOSIS OF RABBITS. By Charles Weiss. *From the Research Laboratories of the Mount Zion Hospital, San Francisco.*

In recent years there has been an increasing tendency to study the mechanism of allergy and immunity in tuberculosis from the viewpoint of the phenomenon of inflammation. Because of the important rôle played by the leukocytic and tissue proteinases in this process, we have studied the behavior of cathepsins in experimental tuberculosis of rabbits. It was observed that there is a marked depression of splenic cathepsin in animals which had been rendered tuberculin positive by repeated injection with a strain of low virulence and then reinfected with a virulent bovine strain of *M. tuberculosis*. Similar depressions in cathepsin were noted in the livers of these animals, but not in the kidneys or lungs. Reinfection with a strain of low virulence gave negative results.

It is of interest to note that the spleens and livers which present decreases in cellular cathepsins are, in Lurie's experiments, the most efficient in destroying virulent tubercle bacilli.

In vitro experiments demonstrated an inhibitory action of the phosphatide fraction of tubercle bacilli on cathepsins. It is suggested, therefore, that the destruction of virulent tubercle bacilli *in vivo* liberates phosphatide which, in turn, inhibits the activity of tissue cathepsins.

The probable relationship of these phenomena to the processes of caseation and softening of tuberculous tissue was discussed.